Eco-evolutionary processes in Caribbean reef fish (*Hypoplectrus* spp)

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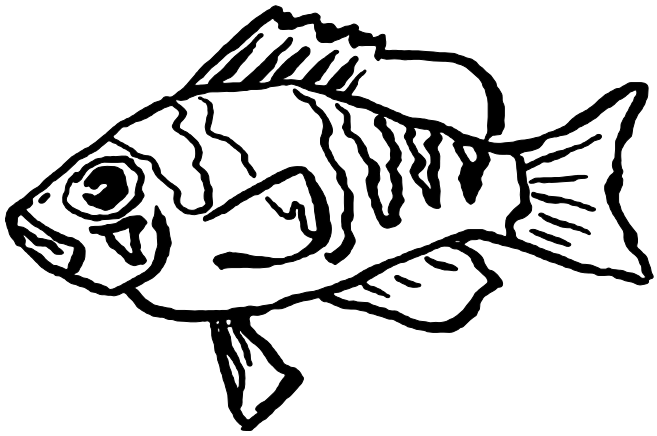

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SUMMARY

How novel traits can arise, spread and persist is a captivating question in evolutionary biology. Here, we embrace a broad and inclusive definition that spans all levels of biological organization, viewing novelties as traits that are new in composition or context of expression relative to previously existing traits. The main objective of my thesis was to investigate the eco-evolutionary processes leading to the spread and diversification of novel traits (namely color polymorphism and the rare mating system of egg trading) in the Caribbean hamlet fish (*Hypoplectrus* spp), one of the few adaptive radiations documented in the marine realm.

The diversity in color within this genus is remarkable: at least 17 species have been described so far, each characterized by differently striking coloration and pattern, but otherwise eco-morphologically largely identical. Color pattern also varies within hamlet species, which adds an interesting level of intraspecific diversity to the already existing inter-species color polymorphism. Given these interesting patterns of intraspecific and interspecific diversity, I first focused on investigating the genomic basis of local adaptation (within species) and compared it to the genomic basis of speciation (between species) using RAD sequencing. Interestingly, I found very similar architectures, characterized by few islands of differentiation against a background sea level of very low levels of differentiation.

I then turned to focus on the ecological and behavioral significance of color pattern at the phenotype level. At least seven hamlet species have been hypothesized to be aggressive mimics of other reef fish species. The aggressive mimicry hypothesis postulates that by resembling other non-predatory reef fishes (similarly-sized and more abundant), the predatory hamlets gain an advantage in the approach and attack of their

prey. I conducted detailed behavioral observations of the butter hamlet (*H. unicolor*), a putative mimic of the foureye butterflyfish (*Chaetodon capistratus*), to quantify variation in aggressive mimicry and mate choice behavior at the individual level. I found that individuals differ consistently in how much they engage in aggressive mimicry behavior, forming two discrete behavioral types, or alternative behavioral phenotypes, that also differ consistently with respect to foraging, territoriality, and mate choice behavior. Pairing observations of these individuals revealed that mating tends to be assortative with respect to behavioral type, suggesting that aggressive mimicry behavior may play an important role in mate choice.

Finally, I focused on the evolution of the rare mating system that the hamlets engage in, known as egg trading. Hamlets are simultaneous hermaphrodites, producing male and female gametes at the same time. They spawn in pairs, where partners take turns offering their eggs for fertilization in exchange for the opportunity to fertilize their partner's eggs. I focused on modeling the evolutionary dynamics that lead to the invasion and stability of egg trading when other mating strategies already exist in a given population of simultaneous hermaphrodites. The model was calibrated with parameters derived from new and long-term field observations of the pairing dynamics of the butter hamlet. The model predicted that under a combination of intermediate encounter rates and high opportunity costs of eggs, egg trading can be the only evolutionary stable outcome to evolve. Although the empirical data provided a realistic sense of egg production and egg senescence rate in a species where egg-trading has successfully evolved and is currently maintained, it also highlighted the need to clearly distinguish the forces leading to the initial establishment of egg trading and the forces underlying its maintenance.

ZUSAMENFASSUNG

Eine fesselnde Frage in der Evolutionsökologie besteht darin, wie neue Merkmale entstehen, sich verbreiten und fortbestehen. Ein Merkmal ist neu, wenn es relativ zu bereits bestehenden Merkmalen in neuer Zusammensetzung oder neuem Zusammenhang ausgeprägt wird. Die vorliegende Arbeit untersucht wie sich ebensolche neuen Merkmale auf dem Genom-, Phänotypen- und Populationslevel verbreiten und diversifizieren. Untersucht wird diese Frage anhand der Evolution von Farbmustern und des ungewöhnlichen Paarungssystems des karibischen Hamletbarsches (*Hypoplectrus* spp), welcher ein seltenes Beispiel für rezente marine adaptive Radiation darstellt. Dieses Genus zeigt eine bemerkenswerte Farbenvielfalt: mindestens 17 Arten wurden bisher beschrieben, jede charakterisiert durch einzigartige Farben und Muster, ökomorphologisch ansonsten aber weitgehend identisch. Die Hamletbarsche bieten ein vielversprechendes System, da das gesamte Spezifizierungsspektrum von genetisch fast ununterscheidbar zu klar abgegrenzten Arten vorhanden ist. Zusätzlich zu diesem inter-spezifischen Farbpolymorphismus entsteht ein weiterer interessanter Aspekt von intraspezifischer Diversität durch variierende Farbmuster auch innerhalb der Arten. Aufgrund dieser intra- und interspezifischen Unterschiede untersuchte ich zunächst mithilfe von RAD sequencing die genomische Basis intraspezifischer lokaler Adaption und verglich diese mit der genomischen Basis der interspezifischen Diversifizierung. Interessanterweise zeigen beide ähnliche Architektur, charakterisisert durch wenige Inseln, (jeweils mit individuellen Loci) vor dem Hintergrund eines sehr geringen Differenzierungslevels.

Im Folgenden untersuchte ich die ökologische und verhaltenswissenschaftliche

Bedeutung der Farbmuster auf Phänotypenlevel. Eine Kombination aus natürlicher Selektion (durch Krypsis und aggressive Mimikry) und sexueller Selektion (durch assortatives Paarungsverhalten) wurde zuvor als Erklärung der Diversifizierung von Farben in dieser Radiation herangezogen. Speziell die Hypothese der aggressiven Mimikry postuliert, dass predatorische Hamletbarsche durch die Ähnlichkeit zu anderen, nicht-predatorischen Rifffischen, ähnlich in Größe und zahlreicher im Auftreten, Vorteile in der Annäherung und im Angriff auf ihre Opfer erlangen.

In meinem zweiten Kapitel führte ich detailreiche verhaltenswissenschaftliche Beobachtungen an Butter-Hamletbarschen (*H. unicolor*), ein potentieller Imitator des Vieraugen-Falterfisches (*Chaetodon capistratus*), durch, um Varianz in der aggressiven Mimikry und bei der Partnerwahl auf Individuenlevel zu quantifizieren. Nach meinen Beobachtungen unterschieden sich Butter-Hamletbarsche konsistent in der Häufigkeit der aggressiven Mimikry, wobei zwei verschiedene Verhaltenstypen, oder alternative Phänotypen zu beobachten sind, welche sich auch bei der Nahrungssuche, Territorialität und Partnerwahl beständig unterschieden. Genauere Untersuchung dieser Beobachtungen ergaben, dass diese Phänotypen assortativ paaren, was eine wichtige Rolle der aggressiven Mimikry in der Partnerwahl nahe legt.

Schlussendlich betrachtete ich die Evolution des seltenen Paarungssystems der Hamletbarsche, welches sich durch Ei-Handel auszeichnet. Hamletbarsche sind simultane Hermaphroditen, welche gleichzeitig männliche und weibliche Gameten ausbilden. Sie laichen in Paaren, wobei die Partner abwechselnd ihre Eier zur Befruchtung anbieten, um im Gegenzug die Möglichkeit zu erlangen die Eier des Partners zu befruchten. In Kapitel drei stelle ich ein Modell vor, welches die Evolutionsdynamik simuliert, die zur Einführung und Etablierung des Ei-Handels in Anwesenheit anderer Paarungsstrategien

in einer Population von simultanen Hermaphroditen führte. Die Parameter dieses Modells wurden durch eigene neu erhobene Langzeitbeobachtungen kalibriert. Das Modell prognostiziert, dass Ei-Handel bei einer Kombination von mittlerer Begegnungsrate und hohen Opportunitätskosten als einzig evolutionär stabiles System entstehen kann. Obwohl die empirisch erhobenen Daten eine realistische Schätzung von Eiproduktions- und Seneszenzraten in einer Art mit erfolgreich etabliertem Ei-Handel erlauben, zeigt der Vergleich von empirischen mit Modelldaten, dass die Mechanismen, die zur ursprünglichen Etablierung eines Paarungssystems wie des Ei-Handels führen unterschieden werden müssen von solchen, die das Fortbestehen eines solchen Systems bedingen.

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I am also grateful for the financial support I got for this research through my PhD scholarship from the International Max Planck Research School for Evolutionary Biology and from the Smithsonian Tropical Research Institute.

Above all, I would like to thank my family for their support and encouragement, and for coming to Kiel to visit me so often, merci toujours! I am also incredibly grateful to Seamus, for not despairing and always supporting me. And of course to my friends, especially Lisa for helping me in the last days of writing (and for translating the abstract!), but also Kosmas (who also helped in the translation!), Anja, Agnes and Tillmann, Miguel, Tana, Oscar, Myriam and Olivia for their great company throughout these three years. Thank you!

CONTRIBUTION OF AUTHORS

All chapters of this thesis were designed and written for peer-reviewed publications. The contribution of each author, including my own, is specified in this section.

Population genomics of local adaptation vs. speciation in coral reef fishes (Hypoplectrus spp, Serranidae)

Oscar Puebla and Sophie Picq designed the study. This study is based on the same dataset presented in the Puebla et al. (2014) study, so DNA extractions and library preparations were conducted by Oscar Puebla in the laboratory of Owen McMillan in the Smithsonian Tropical Research Institute. All analyses were performed by Sophie Picq, with input from Oscar Puebla. Both Sophie Picq and Oscar Puebla wrote the manuscript.

Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population

Sophie Picq and Oscar Puebla designed the study. Sophie Picq collected and analysed the field data, with input from Oscar Puebla. Marco Scotti performed the individual-based model. All authors contributed to the writing.

On the evolution of egg trading in simultaneous hermaphrodites

Oscar Puebla, Jorge Peña, and Georg Nöldeke designed the study. Sophie Picq and Oscar Puebla led discussions to introduce JP and GN to the biological world of egg trading. Georg Nöldeke and Jorge Peña carried out the analytical model analysis. Sophie Picq collected and analyzed the field data with input from OP. All authors contributed to the writing.

DECLARATION

Hereby I declare that,

i. apart from my supervisor's guidance, the content and design of this dissertation is product of my own work. Contributions from coauthors to specific paragraphs are listed in the next section.

ii. this thesis has not been submitted either partially or wholly as part of a doctoral degree to another examination body, and no other materials are published or submitted for publication than indicated in the thesis

iii. the preparation of the thesis has been subjected to the Rules of Good Scientific Practice of the German Research Foundation.

Kiel

Sophie Caroline Picq

GENERAL INTRODUCTION

Understanding how evolutionary novelty can arise, persist, and diversify is a fascinating question that has captivated biologists for decades and remains a research priority today (Darwin 1859; Huxley 1942; Mayr 1959; West-Eberhard 2003; Wagner and Lynch 2010). The concept of evolutionary novelty has been considered "necessarily fuzzy" (Pigliucci 2008) for the many ramifications it entails; definitions are manifold, ranging from the highly restrictive, applying to only a limited number of spectacular structures (*e.g.* Mayr 1963) to the highly inclusive, encompassing any feature of an organism (*e.g.* Arthur 2000). Here, we embrace a broad and inclusive definition that spans all levels of biological organization, viewing novelties as morphological or behavioural traits that are new in composition or context of expression relative to previously existing traits (West-Eberhard 2003; Pigliucci 2008). This definition assumes that the evolution of novelty encompasses three major steps, each governed by different evolutionary forces, namely: trait *origin* (genetic and developmental mechanisms generating a new trait), trait *spread* (the selective context, fitness effect, or adaptive function that causes a trait to increase in frequency or be maintained), and potentially, trait *diversification* (novel trait states, or variations of the new trait, may arise) (Huxley 1942; West-Eberhard 2003; Wagner and Lynch 2010). The third step is 'optional', as not all novelties lead to main diversification events; first, some appear, and do not have evolutionary success (exemplified by the 'weird wonders' of the Cambrian Burgess Shale) or fail to radiate (such as the drilling radula that evolved in Late Triassic carnivorous gastropods to drill holes in the shelves of their prey, and was soon lost after its appearance (Fürsich and Jablonski 1984)). Some novelties may appear and persist without diversification, or there may be a 'macroevolutionary lag' (Erwin 2015) between

formation of novelty and successful diversification (for instance, grasslands did not spread until tens of millions of years after the origin and early diversification of grasses (Strömberg 2005)).

Most-often cited examples of novelties include the shell of turtles (Cebra-Thomas et al. 2005), flight (Prum 2005), flowers (Albert et al. 2002), or the ability of great tits to open bottles of milk (Kothbauer-Hellman 1990). According to our definition, the various jaws of African cichlids (Fryer and Iles 1972), as well as the beak shapes and sizes of Darwin's finches (Grant 1981) and Hawaiian honeycreepers (Amadon 1950), are also key novelties that have played a tremendous ecological role in the diversification of their groups. Moreover, some of the most strikingly diverse phenotypes occurring and remaining at the intraspecific level may also be considered novelties. Also referred to as 'alternative phenotypes', these are traits expressed in the same life stage and population, more frequently than traits considered anomalies or mutations, and not simultaneously expressed in the same individual (West-Eberhard 1986). Classical examples include the morphotypes of ants, and the horned and hornless male phenotypes of some beetle species (Eberhard 1979).

The question of trait *origin* has become the research priority of a whole field of investigation known as "evo-devo" and is outside the scope of the current thesis, which focuses instead on major questions pertaining to trait spread and diversification processes, such as what are the selective forces driving and maintaining novel traits, even in the face of potentially competing pre-existing traits? What are the mechanisms ensuring new traits are not swamped by gene flow and reoccur in future generations? What is the underlying genomic architecture that accommodates and supports novel traits? What are the fitness benefits conferred by novel traits? Are genes or phenotypes the initial target of

evolutionary change? This controversy has been dominated in the 20th century by insights into how gene-frequencies underlie evolutionary change, which led to a strong focus on the direct genetic effects on the fitness of individuals and to the assumption that new traits arise essentially from changes in the genome (Williams 1966; Dawkins 1976; O'Donald 1982; Carroll 2008). Nonetheless, many researchers are now convincingly predicting that environmentally initiated phenotypic change can precede genetic change and facilitate adaptation (West-Eberhard 2003; Allf et al. 2016; Levis and Pfennig 2016; Schneider and Meyer 2017).

Novelties do not originate randomly in time and space: indeed, it has been shown that most successful evolutionary novelties originate in the tropics (Jablonski 1993). This is in line with the latitudinal increase in biological diversity towards the tropics, a fundamental pattern in biogeography and ecology that has been recognized since the 1800s and quantified in the 1950s (Dobzhansky 1950; Fischer 1960) in many different organisms (plants, mammals, fishes), both on continents and in oceans. One such species-rich tropical ecosystem is coral reefs, characterized by high levels of diversity in a marine environment where absolute geographic barriers to gene flow are rare and where many species have a pelagic larval stage with potential for extensive dispersal. These two factors are expected to promote gene flow and thereby limit opportunities for species formation, yet coral reefs are among the most species-rich habitats in the world.

In the case of the often extraordinarily coloured coral reef fishes inhabiting these ecosystems, colour is an obvious trait that has undergone diversification. It has been shown to play an important ecological role in terms of preventing detection by prey, increasing predation success, or reducing detection by predators (e.g. in *Plectrochromis* dottybacks (Cortesi et al. 2015) or in *Plagiotremus* fangblennies (Cheney and Côté

2005)), as well as in reproduction, in terms of species-recognition and mate choice (e.g. in *Chaetodon* butterflyfishes, McMillan et al. 1999).

Among coral reef fishes, the brightly coloured Caribbean hamlets (*Hypoplectrus* spp, Serranidae) offer an outstanding system to investigate how evolutionary novelties arise and spread, for two reasons: first, the diversity of colour within this genus is remarkable: hamlets are one of the few examples of a very shallow adaptive radiation in the marine realm (Puebla 2009), with at least 17 species described so far (figure 1) (a third of which have been described in the past few years (Del Moral Flores et al. 2011; Lobel 2011; Victor 2012; Tavera and Acero 2013)) that differ almost exclusively in terms of colour pattern.

Figure 1 Pictures of the 17 hamlet species described so far. From left to right, first row: *H. unicolor* (butter hamlet), *H. puella* (barred hamlet); second row *H.aberrans* (yellowbelly hamlet), *H. nigricans* (black hamlet), *H. castroaguirrei* (Veracruz white hamlet), *H. chlorurus* (yellowtail hamlet), *H. affinis* (blue-lip hamlet); third row: *H. ecosur* (spotted hamlet), *H. atlahua* (jarocho hamlet), *H. indigo* (indigo hamlet), *H. gemma* (blue hamlet), *H. providencianus* (masked hamlet); fourth row: *H. guttavarius* (shy hamlet), *H. floridae* (Floridian hamlet), *H. maya* (maya hamlet), *H. gummigutta* (golden hamlet), *H. randallorum* (tan hamlet)

Secondly, hamlets have evolved a rare and intriguing mating system: they are simultaneous hermaphrodites, producing functional male and female gametes at the same time, a sexual system shared with only approximately 40 other fish species among all vertebrates (Fischer and Petersen 1987; Cole 1990). Hamlets also engage in a specific behaviour known as egg trading, which is the offering of eggs for fertilization by a partner in exchange for the opportunity to fertilize its partner's eggs. So far, egg trading has only been reported to evolve in Serraninae fishes (Fischer 1980*a*, 1984; Pressley 1981; Petersen 1995; Oliver 1997) and in the genus *Ophryotrocha* of the dorvilleid polychaetes (Sella 1985; Sella et al. 1997; Sella and Ramella 1999; Sella and Lorenzi 2000). Hamlets thus provide a great opportunity to investigate the evolution of novelty from different angles: by focusing on the evolution of their colour polymorphism and on the evolution of their mating system.

Hamlets are distributed in the Gulf of Mexico and the Caribbean, and are well-diverged between these two regions at mitochondrial markers (Victor 2012; Tavera and Acero 2013); yet, within these two regions, they tend to be very closely related genetically (McCartney et al. 2003; Barreto and McCartney 2007; Tavera and Acero 2013; Puebla et al. 2014). Hamlets vary in their distribution and are highly sympatric, with up to nine different morphs found on the same reef (Puebla et al. 2012). All species are very similar from an ecomorphologial perspective: they share the same habitat and are reef-associated predators that feed on small invertebrates and fishes (Randall 1967; Whiteman et al. 2007; Holt et al. 2008). To date, colour pattern is the only trait that consistently differentiates species (Randall 1968; Lobel 2011; Tavera and Acero 2013). Spawning occurs before sunset on a daily basis throughout the year. Sympatric species spawn at the same time and in the same area, within sight of each other. Yet, mating is

strongly assortative with respect to colour pattern, with >98% of spawnings occurring among members of the same species (Fischer 1980*b*; Barreto and McCartney 2007; Puebla et al. 2007, 2012). Apparently, there are no strong intrinsic post-fertilization barriers in the hamlets (Whiteman and Gage 2007) and in the only case where hybrids were bred in aquaria, they appeared intermediate between parental species in terms of colour pattern (Domeier 1994). It is important to note that colour pattern also varies with species, both within, and between locations (Thresher 1978; Aguilar-Perera 2004), which adds an interesting level of intraspecific diversity to the already existing inter-species colour polymorphism in the hamlets.

Colour pattern has been hypothesized to play an important ecological role through crypsis and aggressive mimicry in hamlets, and has likely been shaped in part by natural selection (Puebla 2009). The aggressive mimicry hypothesis in particular postulates that by resembling other non-predatory reef fishes (similarly-sized and more abundant), the predatory hamlets gain an advantage in the approach and attack of their prey (Randall and Randall 1960; Thresher 1978; Puebla et al. 2007; but see Robertson 2013). At least seven hamlet species have been hypothesized to mimic different species of reef fishes (see Chapter II, figure1). In particular, Puebla et al. (2007) provided behavioural evidence of a putative model-mimic relationship between *H. unicolor* and the foureye butterflyfish (*Chaetodon capistratus*). Field observations indicated that *H. unicolor* spent about 10% of its time tracking *C. capistratus*, but did about 50% of all its predatory strikes during that time, suggesting that aggressive mimicry relies not only on the resemblance between the two fish in terms of colour pattern, but also on this very specific behaviour whereby *H. unicolor* actively tracks *C. capistratus* while foraging. Further quantification of this behavioural relationship at the individual level and its link to mate choice within butter

hamlets is detailed in Chapter II.

Colour pattern is also under sexual selection, as mating is strongly assortative with respect to colour pattern. Moreover, an earlier study using a combination of behavioural observations in the wild, individual-based simulations and population genetic analysis showed that in the case of the hamlets, where mate choice is mutual, sexual selection alone can drive assortative mating with respect to colour pattern (Puebla et al. 2012). Interestingly, this suggests that the particular mating system of the hamlets may have been an important factor contributing to their rapid diversification in colour pattern.

This thesis addresses the global questions of novelty spread and diversification, from three different angles and at three different levels of biological organization, using the hamlets as a model system: first, by looking at the *genomic basis* of two processes that drive novelty, namely local adaptation and speciation, among populations and species (chapter I); second, by focusing on intraspecific *phenotypic novelty in behaviour*, and how it may be maintained through the behavioural mechanism of assortative mating, at the individual level (chapter II); and lastly, by looking at how a novel mating system representing a novelty shared among species of a subfamily, may invade and become stable in a population composed of different sexual alternative strategies, by using *evolutionary game dynamics* (chapter III).

THESIS OUTLINE

The main objective of my thesis was to investigate the eco-evolutionary processes leading to the spread and diversification of novel traits (namely color polymorphism and the rare mating system of egg trading) in the Caribbean hamlets, using a combination of different tools in order to gain a broader understanding of the dynamics at play. First, in Chapter I and II, I (and colleagues) investigate the genomic and behavioral variation underlying the adaptive radiation of the hamlets. Second, in Chapter III, we bring together mathematical theory and empirical data to develop an applicable understanding of the necessary conditions for the invasion and maintenance of the rare egg-trading sexual system in simultaneous hermaphrodites.

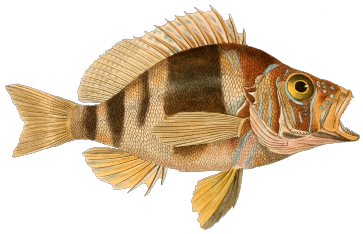
Chapter I focuses on the genomic variation within and among three sympatric species sampled at three repeated populations, using restriction site-associated DNA sequencing. This sampling scheme allowed us to compare the genomic architecture of speciation (differences between species, repeated in three independent locations), to the genomic architecture of local adaptation (differences within species across locations, repeated in three species). This study, entitled "Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp, Serranidae)" has been published in *Ecology and Evolution* in January 2016 and is included here in its final published format.

Chapter II focuses on quantifying variation at the individual level in aggressive mimicry behavior among butter hamlets (*H. unicolor*) and on investigating potential links between this behavior and mate choice, through long-term behavioral observations of aggressive mimicry and mate choice, social network analysis, and individual-based models. Aggressive mimicry has been proposed to play an important role in the

diversification of the hamlet group, and this study allowed us to investigate whether intraspecific variation in this important ecological trait parallels the large-scale patterns of speciation in *Hypoplectrus*. This study, entitled "Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population" has been submitted to *The American Naturalist*, who has just invited us to resubmit a revised version. It appears here in the final form of the submitted manuscript.

Chapter III zooms out of the *Hypoplectrus* radiation to focus on modeling the evolutionary dynamics leading to the invasion and stability of egg trading, a rare mating system in simultaneous hermaphrodites. We address this question with an analytical model that considers encounter rates of individuals and opportunity costs of producing eggs in a population where three mating strategies. The model is calibrated with parameters estimated from extensive field observations of the pairing dynamics of butter hamlets. The integration of the empirical data with the model allowed us to consider aspects of the biology of egg trading that may explain why this mating system is so rare. This study, entitled "On the evolution of egg trading in simultaneous hermaphrodites" appears in the form of a manuscript and is currently still in preparation.

Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp)



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Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp, Serranidae)

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Fish, local adaptation, marine, RAD sequencing, speciation.

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Abstract

Are the population genomic patterns underlying local adaptation and the early stages of speciation similar? Addressing this question requires a system in which (i) local adaptation and the early stages of speciation can be clearly identified and distinguished, (ii) the amount of genetic divergence driven by the two processes is similar, and (iii) comparisons can be repeated both taxonomically (for local adaptation) and geographically (for speciation). Here, we report just such a situation in the hamlets (*Hypoplectrus* spp), brightly colored reef fishes from the wider Caribbean. Close to 100,000 SNPs genotyped in 126 individuals from three sympatric species sampled in three repeated populations provide genome-wide levels of divergence that are comparable among allopatric populations (F_{st} estimate = 0.0042) and sympatric species (F_{st} estimate = 0.0038). Population genetic, clustering, and phylogenetic analyses reveal very similar patterns for local adaptation and speciation, with a large fraction of the genome undifferentiated (F_{st} estimate $\approx$ 0), a very small proportion of F_{st} outlier loci (0.05–0.07%), and remarkably few repeated outliers (1–3). Nevertheless, different loci appear to be involved in the two processes in *Hypoplectrus*, with only 7% of the most differentiated SNPs and outliers shared between populations and species comparisons. In particular, a tropomyosin (*Tpm4*) and a previously identified hox (*HoxCa*) locus emerge as candidate loci (repeated outliers) for local adaptation and speciation, respectively. We conclude that marine populations may be locally adapted notwithstanding shallow levels of genetic divergence, and that from a population genomic perspective, this process does not appear to differ fundamentally from the early stages of speciation.

Introduction

Whether populations are adapted to local conditions and, if so, through what mechanisms are fundamental questions in evolutionary ecology (Williams 1966; Kawecki and Ebert 2004; Savolainen et al. 2013). This is particularly true in the marine environment, where absolute barriers to the movement of organisms are few and planktonic larval stages provide potential for extensive dispersal. Are marine populations able to adapt to local environmental conditions in such a potentially high gene-flow context? This is not only a basic question but also an applied one, as the occurrence of locally adapted marine populations has far-reaching implications for management, conservation, and the ability to cope with global change (Conover et al. 2006; Hauser and Carvalho 2008; Munday et al. 2013).

Common gardens and reciprocal transplants can provide direct evidence of local adaptation. These approaches suggest that local adaptation is not uncommon in marine species, even in the presence of planktonic dispersal, and sometimes at small spatial scales (Sotka 2005; Sanford and Kelly 2011). Nevertheless, such experiments can be challenging to implement in highly mobile or hard-to-breed species, which are both common in the marine environment. In addition, the selective factors involved are not always identified and the specific traits underlying local adaptation as well as their genomic bases are almost universally unknown.

Genome scans provide the opportunity to identify the genetic footprints of local adaptation in natural populations, even in the absence of a priori hypotheses about the selective factors and specific traits involved (Savolainen et al. 2013; Tiffin and Ross-Ibarra 2015). Such studies are starting to accumulate in marine fishes (Lamichhaney et al. 2012; Milano et al. 2014), with the Atlantic cod leading the pack (Bradbury et al. 2013; Hemmer-Hansen et al. 2013; Berg et al. 2015). Although a number of factors unrelated to adaptation can generate false positives in genome scan data (Pérez-Figueroa et al. 2010; Bierne et al. 2011, 2013; Vilas et al. 2012; Lotterhos and Whitlock 2014), all genome scan studies on marine fishes identify candidate loci for local adaptation, with temperature and salinity emerging as usual suspects regarding the selective factors involved.

An important aspect of local adaptation is its potential to initiate, facilitate, or drive speciation (Gavrilets 2003; Kawecki and Ebert 2004; Nosil 2012; Savolainen et al. 2013; Tiffin and Ross-Ibarra 2015), and the ecological hypothesis of speciation (Schluter 2001) specifically postulates that speciation may result as a by-product of local adaptation. Nevertheless, marine local adaptation and speciation are often considered in isolation of each other. Here, we aim to bridge this gap by asking whether the population genomic patterns underlying local adaptation and speciation are comparable. Addressing this question requires a system in which (i) local adaptation and the early stages of speciation can be clearly identified and distinguished, (ii) the amount of genetic divergence driven by the two processes is similar (thereby eliminating the confounding factor posed by divergence when species are more diverged than populations), and (iii) comparisons can be repeated both taxonomically (for local adaptation) and geographically (for speciation).

The hamlets (*Hypoplectrus* spp, Serranidae) constitute just such a system. These reef fishes from the wider Caribbean are known for their striking variation in color pattern (Thresher 1978; Fischer 1980; Domeier 1994; Lobel 2011). Seventeen species have been described to date, which differ essentially in terms of color pattern. A combination of natural selection on color pattern (Thresher 1978; Puebla et al. 2007) and sexual selection (Puebla et al. 2012a) has been put forward to explain the origin and maintenance of species within the radiation. The hamlets are highly sympatric, with up to nine species found on a single reef. The different hamlet species spawn at the same time and in the same areas, often within sight of each other. Nevertheless, spawning is strongly assortative with respect to color pattern, with >98% of spawnings occurring among members of the same species (Fischer 1980; Barreto and McCartney 2007; Puebla et al. 2007, 2012a). Hamlets from the Gulf of Mexico appear to be well diverged (Victor 2012; Tavera and Acero 2013), but species within the Caribbean are extremely similar from a genomic perspective, with F_{st} estimates between sympatric species ranging between zero and 0.080 at microsatellite loci (McCartney et al. 2003; Puebla et al. 2007, 2012a). RAD analysis of three sympatric species repeated in three Caribbean populations confirmed the microsatellite results and identified a very small proportion of SNPs (0.05%) as F_{st} outliers between sympatric species (Puebla et al. 2014). Remarkably, a single SNP was identified as an outlier in repeated populations for the same species pair (repeated outlier). A mini-contig assembled de novo around this SNP mapped uniquely to the genomic region between the *HoxC10a* and *HoxC11a* genes in 10 teleost species, suggesting a possible role for *Hox* gene evolution in hamlet speciation.

Caribbean hamlets also present low level of genetic structure within species, with F_{st} estimates among allopatric populations ranging between 0.006 and 0.047 at microsatellite loci (McCartney et al. 2003; Puebla et al. 2008, 2009). Such low levels of genetic structure are typical of marine species and raise the question as to whether populations are able to adapt to local conditions. Differences in morphology (Thresher 1978; Aguilar-Perera 2004), diet (Whiteman et al. 2007b; Holt et al. 2008), and behavior (O. Puebla, pers. obs.) have been reported between Caribbean hamlet populations, but it is unclear whether these differences are plastic or adaptive, and if they are adaptive, what selective factors might drive them.

Here, we reanalyze the RAD data presented in Puebla et al. (2014), but comparing allopatric populations instead of sympatric species. We hypothesize that if hamlets are locally adapted, outlier loci should occur among populations, and consistently so in the three species (repeated outliers). In addition, if such repeated outliers are present and can be mapped to known genomic regions, their identity may give us a hint as to what selective factors may be important for local adaptation. Finally, we contrast the population genomic patterns underlying local adaptation to the population genomic patterns underlying speciation described in Puebla et al. (2014). Given the distinct natural histories (and hence selective factors) underlying the two processes, we hypothesize that the loci associated with local adaptation should differ from the loci associated with speciation.

Materials and Methods

This study is based on the same dataset presented in Puebla et al. (2014), but comparing allopatric populations instead of sympatric species. In order to allow direct comparisons between local adaptation and speciation, the same methodology used in Puebla et al. (2014) is

followed here. An overview of the methods is provided below and we refer to Puebla et al. (2014) for details. New simulations and new analyses of linkage disequilibrium are described in detail.

Sampling and genotyping

This study is based on nine samples including three sympatric species (the barred hamlet *Hypoplectrus puella*, the black hamlet *Hypoplectrus nigricans*, and the butter hamlet *Hypoplectrus unicolor*) from three locations (Belize, Honduras, and Panama), with 14 individuals per sample (total 126 individuals). This sampling design provides the opportunity to explore the population genomic patterns of local adaptation (between allopatric populations within species) and speciation (between sympatric species) within a single system, and to repeat comparisons both taxonomically (in three species for local adaptation) and geographically (in three populations for speciation).

Libraries were prepared following the restriction site-associated DNA (RAD) sequencing protocol by Etter et al. (2011) and sequenced as detailed in Puebla et al. (2014). In order to compare the results provided by RAD sequencing and microsatellites, microsatellite data from Puebla et al. (2007, 2012a) were reanalyzed for the populations and species considered in this study (10 loci, 50 individuals per sample).

Raw sequences filtering and assembly

Filtering of the raw sequences included the removal of low-quality reads, reads with an ambiguous index or *Sbf*I restriction site, and reads including adapter sequence as detailed in Puebla et al. (2014). Pairs of paired-end reads that matched exactly were filtered out, as these are expected to represent PCR clones in the vast majority of cases.

Reads were assembled de novo using Stacks (Catchen et al. 2011, 2013). The number of raw reads required to form a stack (m) was set to three and the number of allowed nucleotides mismatch between two stacks (M) to two, which is in line with the guidelines provided by Catchen et al. (2013), Ilut et al. (2014), and Mastretta-Yanes et al. (2015). In order to test the robustness of the results to these assembly parameters, the main analyses were rerun with $m = 3$ $M = 3$, $m = 4$ $M = 2$, $m = 5$ $M = 4$, and $m = 10$ $M = 4$.

Population genetic statistics

In order to allow direct comparisons with previous results on speciation, the same moderate filtering used in Puebla et al. (2014) was applied to the dataset unless stated otherwise for specific analyses.

Analyses were also repeated with more stringent filtering and, as indicated throughout the Results, similar genomic patterns were obtained.

Samples were either pooled by location (Belize, Honduras, Panama, $n = 42$ individuals per location), retaining stacks with coverage ≥10x in ≥15 individuals per location in ≥2 locations, or considered individually ($n = 14$ individuals per sample), retaining stacks with coverage ≥10x in ≥5 individuals per group in ≥7 samples. F_{st} were estimated following a standard analyses of variance (ANOVA) approach (Weir and Cockerham 1984) using Genepop version 4.2.1 (Rousset 2008).

Clustering analyses

Clustering analyses (Pritchard et al. 2000) were performed to further explore genetic structure. The same filtering as above was used, but this time considering a single SNP per stack (the first one). The admixture model with correlated frequencies was considered (Falush et al. 2003), and species/location information was not used to preassign individuals to clusters or to improve clustering. K was set from one to 10 and 10 replicate analyses (100,000 MCMC burning steps followed by 100,000 iterations) were run for each value of K. Structure Harvester (Earl and vonHoldt 2012) was used to summarize the results from the 100 runs performed for each analysis. Both ln $\Pr(X|K)$ and the ad hoc statistic ΔK (Evanno et al. 2005) were used to infer the number of clusters present in the dataset.

Genetic structure was further analyzed with different SNP subsets. These were established according to global F_{st} estimates among locations (Fig. 1A), considering the interval above the 90th percentile ($F_{st} \geq 0.0266$, 8038 SNPs), between the 80th and 90th percentiles ($0.0127 \leq F_{st} < 0.0266$, 8467 SNPs), between the 70th and 80th percentiles ($0.0047 \leq F_{st} < 0.0127$, 8424 SNPs), between the 60th and 70th percentiles ($-0.0006 \leq F_{st} < 0.0047$, 8034 SNPs), and below the 60th percentile ($F_{st} < -0.0006$, 33,216 SNPs). This approach should be considered with caution, as there is some circularity in the process of selecting the most diverged SNPs to then explore genetic structure. Here, the most differentiated SNPs were selected to infer roughly how many and which SNPs were consistently differentiated among populations, and compare them with the number and identity of SNPs that were consistently differentiated among species (Puebla et al. 2014).

SNP trees

In order to also adopt a phylogenetic perspective, SNPs were used to generate maximum-likelihood trees. Preliminary

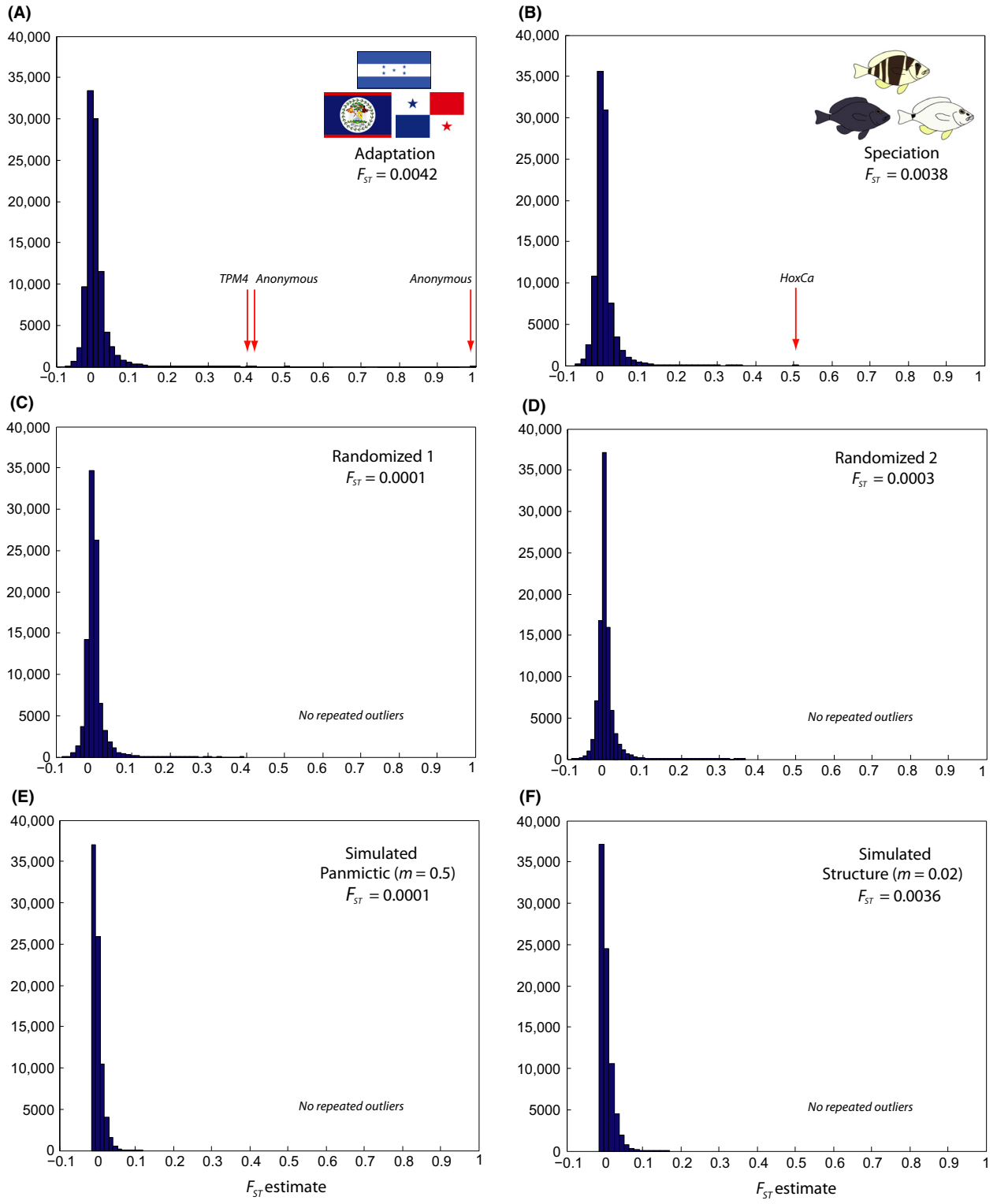

Figure 1. Frequency distribution of individual SNP F_{st} estimates (A) among locations (Belize, Honduras, and Panama, 97,962 SNPs), (B) among species (*Hypoplectrus puella*, *H. nigricans*, and *H. unicolor*, 96,418 SNPs, from Puebla et al. 2014), (C, D) among random groups (95,274 and 95,309 SNPs, respectively), and (E, F) for simulated data (panmictic, migration rate m = 0.5, and structure, migration rate m = 0.02, 80,000 loci in both cases). Repeated outliers highlighted with red arrows.

analyses indicated that individuals with a high proportion of missing data contributed disproportionally to reduce bootstrap support values, so individuals with >20% missing data (mostly black and barred hamlets from Panama due to lower sequencing coverage in these populations) were filtered out. RAxML version 8.0.5 (Stamatakis 2014) was used for these analyses, implementing the GTR+G model with ascertainment bias correction and a rapid bootstrap procedure (Stamatakis et al. 2008) with 100 replicates per run. Analyses were run with the entire SNP dataset, and repeated with the same SNP subsets considered for the clustering analyses. Trees were generated with Dendroscope version 3.2.1 (Huson and Scornavacca 2012).

Linkage disequilibrium network analysis

Linkage disequilibrium network analysis (LDna, Kemppainen et al. 2015) was performed to explore patterns of linkage disequilibrium (LD) in the dataset. Briefly, LDna starts from a matrix of pairwise LD estimates among loci and partitions loci into clusters, in which vertices represent loci, and edges LD values that are above a given threshold. The order in which clusters merge with decreasing LD threshold is represented as a tree where branches correspond to clusters, and nodes merging events. Change in median LD in a cluster at merging is measured by λ, and λ values exceeding the median by a user-defined multiple φ of the median absolute deviation and containing at least $|E|$min edges (user-defined also) identifies outlier clusters. Outlier clusters that do not have any other outlier clusters nested within them are defined as single-outlier clusters (SOCs). We hypothesized that population structure should result in admixture LD when considering the entire dataset, and that clusters of loci in LD should differentiate populations and species.

Preliminary analyses indicated that LDna is sensitive to the occurrence of missing data, rare alleles (present in only one individual per sample), loci with heterozygosities >0.5, and that computation time for the calculation of the initial LD matrix becomes very long for >10,000 loci. LDna analyses were therefore restricted to black and barred hamlets from Honduras and Belize (which had highest sequencing coverage), filtering loci with coverage ≥20x in at least 11 individuals in all populations, removing loci with rare alleles and heterozygosities >0.5 and considering a single SNP per stack, which resulted in 10,734 SNPs. Global F_{st} among the four samples was estimated for each SOC identified, and a DAPC analysis with the four samples as groups was run for each SOC using Adegenet version 1.4-2 (Jombart et al. 2010).

F_{st} outlier analyses

Outlier scans were performed to identify SNPs that may be under selection. Bayescan version 2.1 (Foll and Gaggiotti 2008) was used for these analyses, with default parameters for run length and the prior odds for the neutral model set to 10 (default value) and 100. A locus was considered to be an outlier if it had a q-value <0.2, corresponding to an expected false discovery rate of 20%.

Paired-end reads were used to assemble mini-contigs around the repeated outlier SNPs using Velvet version 1.2.03 (Zerbino and Birney 2008). Matches to the consensus sequences were searched using megablast on the NCBI server (http://www.ncbi.nlm.nih.gov/blast) and Blastn searches to the teleost genomes available on the Ensembl genome browser (Flicek et al. 2014, http://www.ensembl.org/index.html). Blast searches were also performed for the consensus sequence of all stacks that included nonrepeated outlier SNPs.

Randomizations and simulations

In order to complement and better interpret our results, part of the analyses were repeated on randomized and simulated datasets. For the randomizations, the 126 samples were grouped into three random 'species' from three random 'locations' (nine samples total). Simulations were performed with SimuPOP version 1.1.4 (Peng and Kimmel 2005), considering an island model with nine populations of 1000 individuals each. Two scenarios were simulated, one with migration rate $m = 0.5$ ('panmictic') and one with $m = 0.02$ ('structure', which results in levels of genetic structure ($F_{st} \approx 0.004$) that are similar to those observed in the real dataset). Each individual carried 80,000 diallelic unlinked loci with a mutation rate μ of 1E-9. As for the real dataset, 14 individuals were sampled per population. Simulations were repeated three times and sampled 10 times each, resulting in a total of 30 datasets per scenario.

Results

Raw sequences filtering and assembly

A total of 565,253,125 reads of 101 bp each were retained after filtering, corresponding to 83.9% of the raw reads (see Puebla et al. 2014 for details). The main assembly ($m = 3$ $M = 2$) provided an average of 53,811 stacks per sample, with a mean coverage per stack of 31x before SNP filtering. The number of stacks decreased with increasing m and M parameter values, which is expected (Catchen et al. 2013). Nevertheless, similar global F_{st} estimates (0.0042–0.0044) and proportions of outliers

(0.06–0.07%) were provided by the five assemblies with different combinations of *m* and *M* parameters (Table S1).

Population genetic statistics

A total of 53,924 stacks were retained after pooling samples by location and filtering, providing 97,962 SNPs (i.e., 1.8 SNP per stack on average). Population genetic statistics are presented in Table S2. Considering all nucleotides, global diversity (π) and heterozygosity were estimated to 0.00240 and 0.00178, respectively, close to the values of 0.0036 and 0.00187 reported for sticklebacks (Hohenlohe et al. 2010). Global F_{st} among the three locations was estimated to 0.0042 when considering all SNPs. Close estimates of 0.0045, 0.0044, and 0.0039 were obtained when considering a single SNP per locus (the first one), removing loci with rare alleles (present in only one individual per location), or applying more stringent filtering (loci present in ≥32 individuals per location instead of 15), respectively. The distribution of SNP F_{st} estimates presented a sharp mode close to zero and a shallow tail extending to a value of one (Fig. 1A). F_{st} among the three locations was estimated to 0.0063 for *H. unicolor*, 0.0065 for *H. puella*, and 0.0131 for *H. nigricans*. Microsatellite data from the same populations provided close F_{st} estimates of 0.0034 for all species, 0.0032 for *H. puella*, and 0.0084 for *H. nigricans* (Table 1).

When considering the nine samples independently, a total of 31,059 stacks were retained after filtering, providing 55,195 SNPs. F_{st} estimates among populations ranged between 0.0053 (*H. puella* Belize/Honduras) and 0.0330 (*H. nigricans* Belize/Panama, Table 1). Microsatellite data provided F_{st} estimates that ranged between 0.0011 (*H. unicolor* Honduras/Panama) and 0.0132 (*H. nigricans* Belize/Panama (Table 1). We note that sample sizes were relatively low for the RAD data, with a mean *n* of 17–25 per pairwise comparison (vs. 100–108 for microsatellites).

Clustering analyses

The clustering analyses are summarized in Figures 2 and S1. Using the entire dataset (41,690 SNPs), ln Pr($X|K$) was systematically higher for $K = 1$ than for any other value of K in the 10 replicate runs. Nevertheless, the black hamlets from Belize – the most differentiated sample according to the RAD and microsatellite F_{st} estimates – tended to form a distinct cluster in some runs with $K = 2$. This pattern became consistent when removing loci with rare alleles (present in only one individual per location), in which case $K = 2$ was identified as the best clustering solution (Fig. S2).

The SNP subsets from the 90th–100th, 80th–90th, and 70th–80th F_{st} percentiles provided strong evidence of clustering. The highest mean ln Pr($X|K$) corresponded to $K = 3$ (90th–100th and 80th–90th percentiles) and $K = 2$ (70th–80th percentile). In each case, the ΔK statistic presented a clear peak at these K values, and the 10 replicate runs provided almost exactly identical groupings (including the three 'misassigned' samples), although different seed numbers were used for each run. For the 90th–100th and 80th–90th percentiles, the three clusters corresponded to the three locations (Fig. 2). For the 70th–80th percentile, the two clusters differentiated the Honduras samples from the Belize and Panama samples. No clustering

Table 1. F_{st} estimates among Belize, Honduras, and Panama in *Hypoplectrus puella*, *H. nigricans*, and *H. unicolor* at 10 microsatellite loci, 97,962 SNPs, and at the three repeated outliers identified in this study. *n* sample size, n/a data not available, – coverage below filtering criteria for these SNPs in these populations.

			F_{st} estimate (sample size)				
Species	Location		10 μ satellite loci	97,962 SNPs	SNP 39,894 (*Tpm4*)	SNP 55,313 (anonymous)	SNP 38,220 (anonymous)
All species	All locations		0.0034 ($n = 418$)	0.0042 (mean $n = 79.5$)	0.3827 ($n = 92$)	0.4146 ($n = 89$)	1.0000 ($n = 43$)
H. puella	All locations		0.0032 ($n = 154$)	0.0065 (mean $n = 29.3$)	0.3485 ($n = 33$)	0.5178 ($n = 28$)	1.0000 ($n = 20$)
H. nigricans	All locations		0.0084 ($n = 156$)	0.0131 (mean $n = 27.9$)	0.4932 ($n = 26$)	0.4162 ($n = 26$)	1.0000 ($n = 14$)
H. unicolor	All locations		n/a	0.0063 (mean $n = 26.1$)	0.3875 ($n = 31$)	0.5366 ($n = 30$)	1.0000 ($n = 20$)
H. puella	Belize	Honduras	0.0021 ($n = 100$)	0.0050 (mean $n = 25.2$)	0.4933 ($n = 27$)	0.5178 ($n = 28$)	1.0000 ($n = 15$)
H. nigricans	Belize	Honduras	0.0059 ($n = 102$)	0.0135 (mean $n = 24.3$)	0.4933 ($n = 26$)	0.4162 ($n = 26$)	1.0000 ($n = 14$)
H. unicolor	Belize	Honduras	n/a	0.0092 (mean $n = 15.2$)	0.7108 ($n = 17$)	0.8674 ($n = 18$)	1.0000 ($n = 14$)
H. puella	Honduras	Panama	0.0046 ($n = 104$)	0.0222 (mean $n = 19.0$)	0.1871 ($n = 20$)	–	0.0000 ($n = 12$)
H. nigricans	Honduras	Panama	0.0059 ($n = 105$)	0.0361 (mean $n = 18.4$)	–	–	–
H. unicolor	Honduras	Panama	0.0011 ($n = 108$)	0.0090 (mean $n = 17.2$)	0.1035 ($n = 21$)	0.3970 ($n = 18$)	0.0000 ($n = 13$)
H. puella	Belize	Panama	0.0056 ($n = 104$)	0.0213 (mean $n = 18.2$)	0.0360 ($n = 19$)	–	1.0000 ($n = 13$)
H. nigricans	Belize	Panama	0.0132 ($n = 105$)	0.0493 (mean $n = 17.1$)	–	–	–
H. unicolor	Belize	Panama	n/a	0.0069 (mean $n = 20.1$)	0.3545 ($n = 24$)	0.2597 ($n = 24$)	1.0000 ($n = 13$)

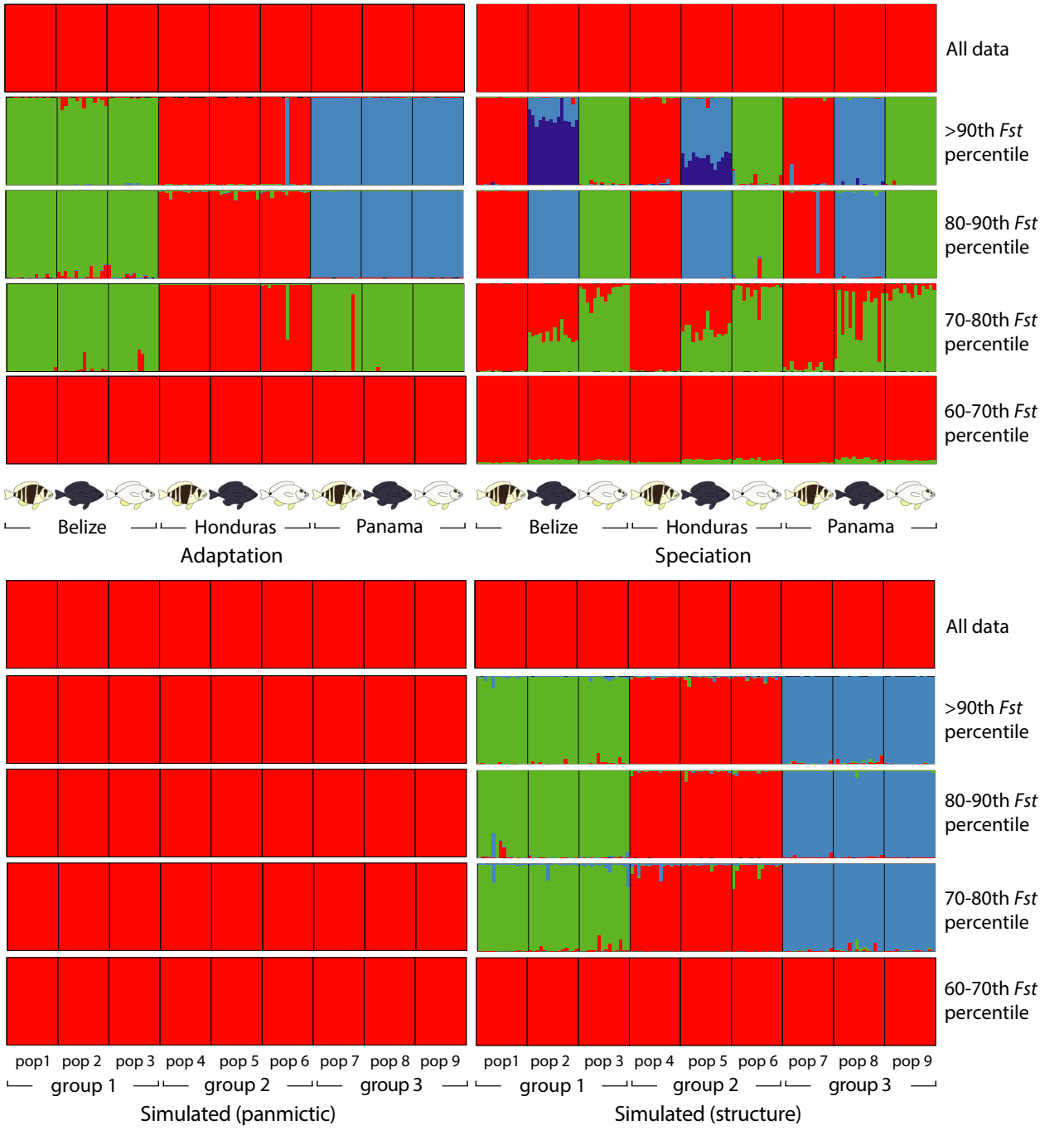

Figure 2. Clustering results for adaptation (among populations, Belize, Honduras, and Panama), speciation (among species, *Hypoplectrus puella*, *H. nigricans*, and *H. unicolor*, from Puebla et al. 2014), and simulated data (panmictic, migration rate $m = 0.5$, and structure, migration rate $m = 0.02$). In each case, the entire dataset (~40,000 SNPs) is presented above, followed by the SNPs above the 90th F_{st} percentile, between the 80th and 90th F_{st} percentiles, between the 70th and 80th F_{st} percentiles, and between the 60th and 70th F_{st} percentiles (~8000 SNPs in each case). Details in Figure S1.

was found with the SNPs from the 60th–70th and 0–60th percentiles. Similar patterns were obtained with more stringent filtering (loci present in ≥32 individuals per species instead of 15, data not shown).

SNP trees

A tendency to group samples by location and species was apparent when considering the entire dataset (Fig. 3), but

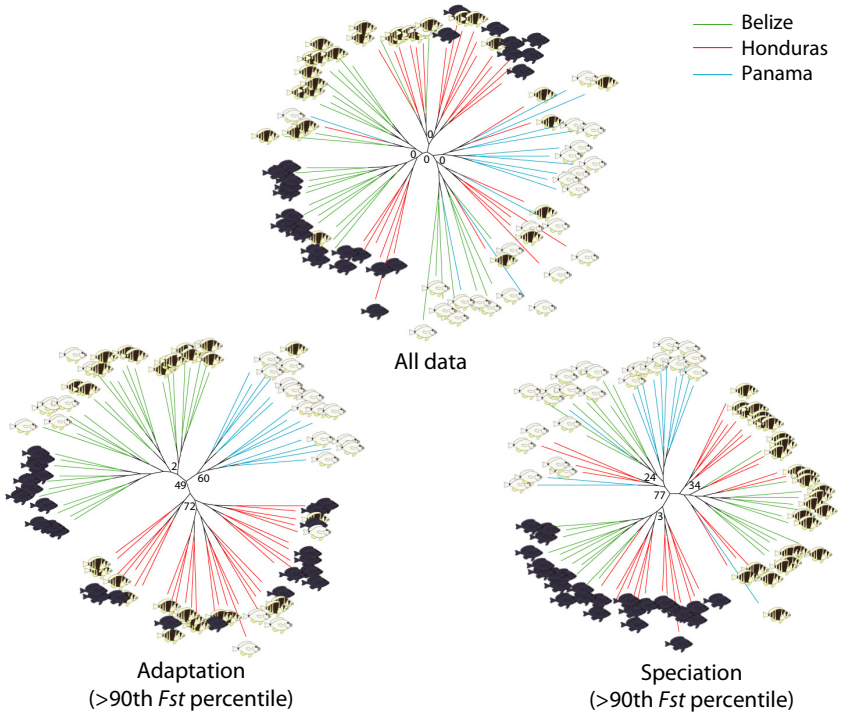

Figure 3. Maximum-likelihood SNP trees for all data, adaptation (among populations, Belize, Honduras, and Panama, SNPs above the 90th F_{st} percentile), and speciation (among species, *Hypoplectrus puella*, *H. nigricans*, and *H. unicolor*, SNPs above the 90th F_{st} percentile, from Puebla et al. 2014). Bootstrap values within groups not shown.

the central node had a bootstrap support value of zero. The SNP subset from the 90th–100th F_{st} percentile grouped samples by location with a bootstrap support value of 49. The SNP subsets from the 80th–90th, 70th–80th, and 60th–70th percentile and below the 60th percentile did not reveal any clear phylogenetic signal, with trees similar to these obtained with the entire dataset (data not shown).

Linkage disequilibrium network analysis

A small proportion of SNPs (249 out of 10,734) presented LD values ≥ 0.8, the large majority of which involved a single pair of loci (Fig. S3). These may be on flanking regions of the same restriction site, as a single SNP per stack was used for these analyses. Larger clusters emerged, grew, and merged at lower LD values. Five single-outlier clusters (SOCs) were identified with φ and $|E|$min set to four and 16, respectively (Fig. 4), and these same SOCs were also detected with various combinations of φ and $|E|$ min (data not shown). The SOCs contained between nine and 43 loci each (total 127), representing 1.2% of the SNPs included in the analysis. Two of them (1149 and 1030) did not appear to distinguish the four samples, with global F_{st} estimates among samples of 0.0027 and −0.0191, respectively. SOC 1030 consisted of relatively tightly linked SNPs (median LD = 0.6 vs. ≤0.2 for the other four SOCs), which may reflect physical linkage (possibly an inversion). The other three SOCs (471, 684, 923) presented higher F_{st} estimates among the four samples (0.0102, 0.0588, and 0.0253, respectively) and diffuse linkage, that is, with a number of edges close to the number of loci. They tended to distinguish the black hamlets from Belize (the most differentiated sample) along the first DAPC axis and the barred hamlets from Honduras (471), barred hamlets from Belize (684), and black hamlets from Honduras (923) along the second axis, suggesting that these SOCs result from admixture LD.

F_{st} outlier analyses

A total of 107 outliers were identified, with the prior odds for the neutral model set to 10, which represents 0.07% of the SNPs analyzed (Table 2). Three of these (38,220, 55,313, and 39,894) were identified in more than one species (repeated outliers) and all of them were 'triple repeated outliers', that is, identified in *H. puella*, *H. nigricans*, and *H. unicolor* independently. Similar results (and the same repeated outliers) were obtained when running the F_{st} outlier analyses globally for each species instead of individually for each population pair (data not shown). Individual F_{st} estimates at the three outlier loci are highlighted in Figure 1A and detailed in Table 1. They were generally high, with global F_{st} estimates among populations within species ranging between 0.348 and one. The latter F_{st} estimate of one corresponded to a SNP on locus 38,220 that was fixed in Belize (C/C) versus Honduras and Panama (G/G) in the three species. A total of 19

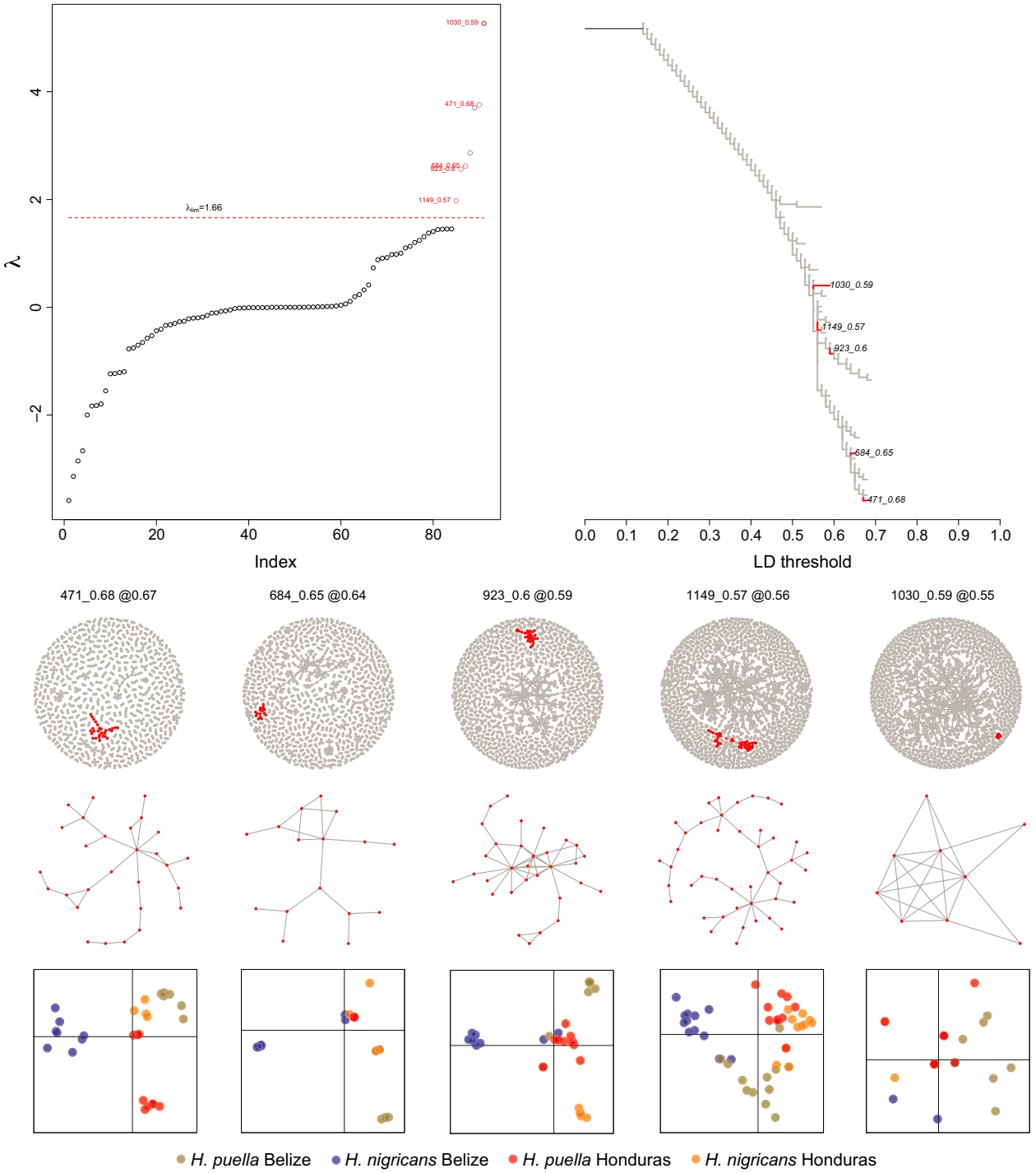

Figure 4. Results of the LDna analyses with $\varphi = 4$ and $|E|\min = 16$. Five single-outlier clusters (SOCs) were identified (in red). Two of them (1149 and 1030) did not appear to distinguish among the four samples, with global F_{st} estimates of 0.0027 and −0.0191, respectively. SOC 1030 consisted of tightly linked SNPs (median LD = 0.6 vs. ≤0.2 for the other four SOCs), which may reflect physical linkage (e.g., an inversion). The other three SOCs (471, 684, 923) presented higher F_{st} estimates among the four samples (0.0102, 0.0588, and 0.0253, respectively) and diffuse linkage (i.e., with a number of edges close to the number of loci). They tended to distinguish the black hamlets from Belize (the most differentiated sample) along the first DAPC axis and the barred hamlets from Honduras (471), barred hamlets from Belize (684), and black hamlets from Honduras (923) along the second axis, suggesting that these SOCs result from admixture LD.

Table 2. Results of the F_{st} outlier analyses between Belize, Honduras, and Panama in *Hypoplectrus puella*, *H. nigricans*, and *H. unicolor*.

				N. (*n*) and ratio (%) of outliers			
				Prior odds=10		Prior odds=100	
Species	Location 1	Location 2	N. loci	*n*	%	*n*	%
H. puella	Belize	Honduras	37,819	22	0.06	4	0.01
H. nigricans	Belize	Honduras	36,256	29	0.08	3	0.01
H. unicolor	Belize	Honduras	15,802	17	0.11	5	0.03
H. puella	Honduras	Panama	10,453	7	0.07	1	0.01
H. nigricans	Honduras	Panama	2145	1	0.05	0	<0.05
H. unicolor	Honduras	Panama	16,492	6	0.04	2	0.01
H. puella	Belize	Panama	10,293	6	0.06	0	<0.01
H. nigricans	Belize	Panama	2073	0	<0.05	0	<0.05
H. unicolor	Belize	Panama	27,847	19	0.07	4	0.01
		Total	159,180	107	0.07	19	0.01

outliers were identified with the prior odds for the neutral model set to 100, which represents 0.01% of the SNPs analyzed. Loci 38,220, 55,313, and 39,894 were identified as outliers here again, as well as in the other assemblies (Table S1). All loci were not included in all analyses as they were below the minimum coverage threshold in some populations (e.g., Table 1). Nevertheless, 78% of the outlier SNPs identified in one species were also considered in at least one other species, indicating that the small number of repeated outliers is not mainly due to a lack of coverage.

A mini-contig of 467 bp and mean coverage of 1033x was obtained for the repeated outlier locus 39,894. The consensus sequence mapped uniquely to an intron in the *Tpm4* gene in five teleosts, with E-values ranging between 5E-25 and 2E-05 (Table 3). Similar blast searches for the other two repeated outlier loci (38,220 and 55,313) did not return strong hits. Blast hits of the nonrepeated outliers are presented in Table S3. Interestingly, one nonrepeated outlier (28,418) mapped to the same *Tpm4* locus identified above in several teleosts. The strongest hit was to the stickleback genome (2E-31), to an intron situated 204 bp from exon 8 and 4826 bp from the repeated outlier.

Randomizations and simulations

Two randomized datasets are illustrated in Figure 1C, D. Global F_{st} were estimated to 0.0001 and 0.0003, respectively, as opposed to 0.0042 for the real dataset. The distributions of SNP F_{st} estimates were similar to the real dataset (Fig. 1A), but slightly narrower and with a shorter tail. With the prior odds for the neutral model set to 10, a total of seven and 21 outliers (0.003 and 0.005% of the SNPs analyzed) were identified for each randomization, respectively, and no repeated outliers were found. For the 'panmictic' scenario ($m = 0.5$), the simulations provided global F_{st} estimates ranging between zero and 0.0005 (mean = 0.0002) and no F_{st} outliers. For the 'structure' scenario ($m = 0.02$), the simulations provided global F_{st} estimates ranging between 0.0031 and 0.0037 (mean = 0.0035) and 67 outliers, representing 0.02% of all the loci considered, and no repeated outliers. An example of each scenario is illustrated in Figure 1E, F. Results of the clustering analyses on the simulated datasets are illustrated in Figures 2 and detailed in S1. No clustering was observed in the 'panmictic' scenario, even when considering the most differentiated SNPs, but clustering patterns similar to these observed in the real data were provided by the 'structure' scenario (Figs. 2 and S1).

Discussion

By specifically targeting the lower end of the 'speciation continuum' (Seehausen et al. 2014), our sampling design provided the opportunity to not only explore the population genomic patterns of local adaptation (among allopatric

Table 3. Results of the blast searches for the consensus sequence of the mini-contig containing the repeated outlier SNP 39,894.

Species	Alignment length (bp)	Identity (%)	E-value	Annotation
Three-spined stickleback (*Gasterosteus aculeatus*)	469	62	5E-25	*tpm4* (intron, 1217 bp from exon 3)
Nile tilapia (*Oreochromis niloticus*)	361	65	5E-23	*tpm4* (intron, 1320 bp from exon 3)
Southern platyfish (*Xiphophorus maculatus*)	134	75	8E-16	*tpm4* (intron, 1502 bp from exon 3)
Spotted green pufferfish (*Tetraodon nigroviridis*)	103	78	2E-11	*tpm4* (intron, 724 bp from exon 3)
Japanes pufferfish (*Takifugu rubripes*)	75	76	2E-05	*tpm4* (intron, 1217 bp from exon 3)

populations) in three hamlet species but also contrast them to the population genomic patterns of speciation (among sympatric species, Puebla et al. 2014). The data revealed very similar levels of genomic divergence (F_{st} estimate = 0.0038–0.0042), F_{st} distributions (Fig. 1), proportions of F_{st} outliers (0.05–0.07%), and numbers of repeated outliers (1–3) for the two processes. In both cases, about 20% and 10% of the most differentiated SNPs distinguished populations and species consistently when considered together in the clustering and phylogenetic analyses, respectively (Figs. 2, 3). These results parallel the population genetic patterns reported in other recently diverged taxa such as East African cichlids (Seehausen et al. 2008; Wagner et al. 2012), Darwin's finches (De Leon et al. 2010), stick insects (Nosil et al. 2012), and the rough periwinkle (Ravinet et al. 2015), where divergence among populations within species or ecotypes can be comparable to divergence among species or ecotypes. Nevertheless, no other study that explicitly contrasts the population genomic patterns along these two axes of divergence comes to mind.

In hamlets, of the 32,681 most diverged SNPs (above the 80th F_{st} percentile), only 7% were shared between populations and species comparisons. This pattern was equally true of outlier loci where, again, only 7% of the F_{st} outliers were shared between populations and species comparisons. In the same line, the three repeated outliers identified among populations differed from the single repeated outlier previously identified among species (Puebla et al. 2014). Different sets of loci appear therefore to be involved in local adaptation and speciation in *Hypoplectrus*, suggesting that genomes are diverging largely independently between allopatric populations versus sympatric species. This may be expected, given the nature of the two processes. Sympatric hamlet species are clearly differentiated in terms of color pattern, but are otherwise morphologically and ecologically extremely similar. Color pattern has been identified as an important trait for mate choice (Domeier 1994; Puebla et al. 2007, 2012a) and aggressive mimicry (Randall and Randall 1960; Thresher 1978; Puebla et al. 2007) in the group, and sympatric species are reproductively isolated from a behavioral perspective by strong assortative mating (Fischer 1980; Barreto and McCartney 2007; Puebla et al. 2007, 2012a). Nevertheless, gene flow is possibly ongoing through the rare hybrid spawnings observed in the field (<2% based on extensive observations), as no intrinsic incompatibilities have been observed in hybrid larvae (Whiteman and Gage 2007a).

Within species, allopatric populations present more subtle differences in morphology, diet, and behavior (Thresher 1978; Aguilar-Perera 2004; Whiteman et al. 2007b; Holt et al. 2008; Puebla et al. 2008), with gene flow occurring through larval dispersal. Fertilization is external in the hamlets and both eggs and larvae are planktonic, with a pelagic larval duration that varies between 2 and 3 weeks (Domeier 1994; B. Victor, pers. comm.), allowing for substantial gene flow among distant locations.

Consistent with this expectation, we observed shallow levels of genetic structure in the hamlets, with a global F_{st} estimate of 0.0042 (0.0063 in *H. unicolor*, 0.0065 in *H. puella*, and 0.0131 in *H. nigricans*) among populations separated by >500 kilometers. Slightly lower F_{st} estimates are provided by microsatellites for the same species and populations (0.0034 global, 0.0032 in *H. puella*, and 0.0084 in *H. nigricans*), which is consistent with the higher diversity and larger sample size of the microsatellite dataset. The results are also consistent with the shallow Caribbean-wide genetic structure reported for *H. puella* using microsatellites (F_{st} estimate = 0.0049, Puebla et al. 2009). Such low levels of population structure are common in marine species and are not surprising, given the life history of the hamlets. Considering patterns of genetic isolation by distance in *H. puella* and *H. nigricans*, we previously estimated a mean dispersal distance of 2–20 km for *Hypoplectrus* (Puebla et al. 2009, 2012b). Moreover, with an average census density of one adult per 150 m^2 of reef in the three species and populations sampled in this study (O. Puebla, unpubl. data) and a simultaneous hermaphroditic mating system that implies a demographic sex ratio of 1:1 (Fischer 1981), the hamlets may have relatively large effective population sizes, which would contribute to maintain low levels of genetic structure. In agreement with the shallow genetic structure reported here, low levels of admixture linkage disequilibrium were observed, with <0.7% of SNPs involved in small and diffuse linkage clusters (Fig. 4).

Local adaptation

The distribution of individual SNP F_{st} estimates indicates that a large fraction of the genome is undifferentiated among populations, with 64% of estimates <0.001 and a sharp mode close to zero (Fig. 1A). Accordingly, it is not surprising to observe no clear structure in the clustering and phylogenetic analyses when considering the entire dataset. Nonetheless, a tendency to group samples by populations and species is apparent in the phylogenetic analyses (Fig. 3A), and the most differentiated sample (the black hamlets from Belize) can be distinguished in the clustering analyses when removing rare alleles (Fig. S2). Thus, part of the genome appears to be differentiated among populations and species. This is further suggested by the long tail of the F_{st} distribution, which goes up to a value of one (Fig. 1A), versus 0.120 and

0.394 in the simulated (panmictic) and randomized datasets, respectively (Fig. 1C, E).

When considered together, the 20% and 10% most differentiated SNPs distinguish the three populations consistently for all species in the clustering and phylogenetic analyses, respectively (Figs. 2, 3). Simulations suggest that such a signal is not expected in the absence of genetic structure (Fig. 2), but we advise caution when interpreting patterns provided by the most diverged SNPs, as there is some circularity in the process of selecting these SNPs to then explore genetic structure, and some signal may result from this procedure with real data, even in the absence of genetic structure (e.g., Fig. S1, randomized dataset). This approach is therefore best suited to explore existing population genetic structure rather than to infer whether or not there is structure. In our case, it is clear from the microsatellite and RAD dataset that there are small differences among populations and species (Table 1). In this context, the most differentiated SNPs were selected to infer roughly what proportion of SNPs were consistently differentiated among populations, and compare them with the proportion and identity of SNPs that were consistently differentiated among species. Another situation in which this approach may be useful is to assign samples to populations when genetic structure is low (e.g., Benestan et al. 2015).

The occurrence of F_{st} outliers provides another line of evidence that part of the genome is differentiated. A total of 107 outliers were identified, representing 0.07% of the SNPs analyzed. Among these, three were identified as repeated outliers in *H. puella*, *H. nigricans*, and *H. unicolor* independently. In contrast, ≤21 outliers and no repeated outliers were found in the randomized and simulated (panmictic) datasets. Two of the three repeated outliers did not map to any known sequence, which illustrates the limitations of RAD sequencing as a tool to identify candidate genes in the absence of a reference genome. On the other hand, one repeated outlier mapped uniquely to an intronic region of the *Tpm4* gene in five teleosts (Table 3). In addition, another nonrepeated outlier also mapped to *Tpm4*, about 5000 bp from the repeated outlier in the stickleback genome. The identification of *Tpm4* as an F_{st} outlier in three hamlet species and at two loci independently suggests that it may be under selection and that it may play a role in local adaptation.

Tpm4 as a candidate gene for local adaptation?

Tpm4 codes for tropomyosin, a ubiquitous two-stranded α-helical coiled coil protein that is best known for its role in muscle contraction, but that is also present in nonmuscle cells in association with actin filaments (Perry 2001). Tropomyosin genes are highly conserved among vertebrates and six of them, including two *Tpm4* genes, have been identified in the Japanese pufferfish (*Takifugu rubripes,* Toramoto et al. 2004). Our repeated outlier (as well as the nonrepeated outlier) mapped exclusively to one of them in all the teleost genomes surveyed, suggesting that the assembly did not merge paralogs for this RAD locus.

Tpm4 has been shown to be associated with diet-induced plasticity in the pharyngeal jaw apparatus of the East African cichlid *Astatoreochromis alluaudi* (Gunter et al. 2013). It is tempting to speculate that the high levels of divergence found in *Tpm4* may be associated with local adaptation to different prey types in Belize, Honduras, and Panama. The hamlets are predators, with a diet that includes small shrimps, crabs, fishes, mysids, stomatopods, isopods, and polychaetes (Randall 1967), and a stomach content analysis including populations from Belize and Honduras evidenced significant differences in prey composition between populations (Whiteman et al. 2007b). Nevertheless, it is unclear to what extent these shifts translate into prey hardness differences that may drive similar effects to what is observed in East African cichlids. Temperature constitutes another, maybe more likely, potential selective factor that may act on tropomyosin through its effect on muscle function. This is particularly relevant for ectotherms, and *Tpm4* has been experimentally shown to be upregulated in skeletal muscle of the common carp (*Cyprinus carpio*) when exposed to cold temperatures (Gracey et al. 2004). Cold-water fronts associated with the southerly extension of the North American high-pressure system have been shown to occur yearly between December and February at the specific location where our Belize samples were collected (Koltes and Opishinki 2009). In this context, it is interesting to note that the *Tpm4* outliers (as well as the unidentified outlier with a F_{st} of one) were identified in pairwise comparisons involving Belize specifically (Belize-Honduras and Belize-Panama). We hypothesize that the outlier signal observed at the *Tpm4* locus is linked to local adaptation to periodic episodes of low temperatures in Belize. Fine mapping of the association between *Tpm4* and population differences is needed to refine this hypothesis and establish to what extent the high levels of genetic differentiation observed in *Tmp4* are due to reduced gene flow (Wu 2001) or low diversity (Cruickshank and Hahn 2014) in this region of the genome.

False positives or parallel adaptation?

Among the 107 F_{st} outliers identified, three were found repeatedly in the three species, suggesting that they might be under selection and possibly involved in local adaptation.

Sequencing coverage at these three loci (41x, 49x, and 88x) was substantially higher than the mean coverage of 31x, suggesting that high divergence does not result from allelic dropout (Gautier et al. 2013). Nevertheless, the significance of the remaining 104 outliers is more open to interpretation. On one hand, nonrepeated outliers may be false positives, a well-known issue in genome scans (Pérez-Figueroa et al. 2010; Vilas et al. 2012; Lotterhos and Whitlock 2014). Our RAD data, assembled de novo and filtered with moderate stringency, surely contain genotyping errors, null alleles, and under- or overmerged loci, all of which are expected to bias downstream analyses (Arnold et al. 2013; Davey et al. 2013; Gautier et al. 2013). Only 19 outliers were detected when applying more stringent parameters in the F_{st} outlier test, and 0.02% of the SNPs analyzed were identified as outliers in the simulated data with structure but no selection (vs. 0.07% in our dataset). This suggests that part of the nonrepeated outliers, possibly as many as 30% of them, may be false positives.

On the other hand, there are reasons to believe that at least a fraction of the nonrepeated outliers are real. First of all, the fact that our global F_{st} estimates are consistent with microsatellite data from the same species and populations and the relatively low levels of heterozygosity of the hamlets suggests that our F_{st} estimates are not disproportionately inflated by the occurrence of null alleles. In addition, the shallow levels of genomic structure reported here provide a very favorable scenario for the detection of loci under divergent selection (Pérez-Figueroa et al. 2010). Finally, it is worth noting that filtering also introduces biases in the data (Arnold et al. 2013; Gautier et al. 2013; Huang and Knowles 2014; Mastretta-Yanes et al. 2015), rendering the solution potentially as problematic as the problem itself. In sum, it is likely that part of the nonrepeated outliers might be real, and that parallel adaptation is occurring in the hamlets. The high proportion of nonrepeated outliers identified among populations reflects the patterns observed among hamlet species (Puebla et al. 2014) and *Littorina* ecotypes (Ravinet et al. 2015), suggesting that parallel evolution may be common in the sea.

Concluding remarks

It is important to keep in mind that hamlets can be more diverged than the populations and species considered in this study, and that the distinction between local adaptation and speciation becomes blurred as populations and species diverge. For example, the hamlets from the Gulf of Mexico appear to be well diverged from similarly patterned Caribbean hamlets, and have been recently described as distinct species (Victor 2012; Tavera and Acero 2013). In this case, local adaptation to the specific conditions of the Gulf of Mexico and Caribbean may have contributed more to species divergence than color pattern. Within the Caribbean, some species such as the Maya hamlet (*Hypoplectrus maya*) or the masked hamlet (*Hypoplectrus providencianus*) present both distinct color patterns and high levels of endemism, suggesting that local adaptation and color pattern may have both played a role in species divergence. Ultimately, whether divergence is considered within the framework of local adaptation or speciation may reflect more a question of perspective and levels of divergence than a fundamental difference between the two processes. We conclude that marine populations may be locally adapted notwithstanding very shallow levels of genomic divergence, and that from a population genomic perspective, this process does not differ fundamentally from the early stages of speciation.

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Data Accessibility

RAD demultiplexed sequence data, SNP genotype calls and mini-contig sequences: Dryad DOI: doi: 10.5061/dryad.nt722.

Conflict of Interest

None declared.

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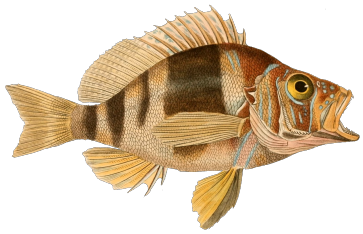
Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Clustering results for adaptation (between Belize, Honduras and Panama), simulated data (panmictic, migration rate $m = 0.5$ and structure, migration rate $m = 0.02$), and randomized data.
Figure S2. Clustering pattern obtained with $K = 2$ when considering all the data and removing rare alleles (present in only one individual per location).
Figure S3. Patterns of linkage disequilibrium among the 10,734 SNPs considered in the LD analysis.
Table S1. Summary of the five assemblies with different combinations of m (stack depth) and M (mismatch) parameters.
Table S2. Mean number of individuals sampled per site, observed and expected heterozygosity, nucleotide diversity (π) and F_{is} in the three locations considered in this study.
Table S3. Highest blast hits for the stacks containing non-repeated outlier SNPs.

CHAPTER II

Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population



Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population (*submitted manuscript*)

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Abstract

What role may animal personality play in speciation and adaptive radiation? Here, we address this question in the butter hamlet (*Hypoplectrus unicolor*), a simultaneously hermaphroditic reef fish from the wider Caribbean, with aggressive mimicry behavior as the focal trait. Aggressive mimicry is of particular interest in the context of speciation in the hamlets because it has been proposed to play a key role in the *Hypoplectrus* radiation. Individuals from a natural population in Panama were tagged and their diurnal and spawning behaviors observed over two years for a total of 159 hours. The data indicate that aggressive mimicry behavior differs consistently among individuals and forms two discrete behavioral types that also differ with respect to foraging behavior and territoriality. In addition, spawning observations indicate that mating tends to be assortative with respect to behavioral type, providing a link between aggressive mimicry, personality and reproductive isolation at the population level and a parallel with the large-scale patterns of speciation and adaptive radiation in *Hypoplectrus*. These results indicate that the traits that characterize adaptive radiations can vary within populations in the form of animal personalities—even in the absence of morphological differences—and that mating can be assortative with respect to such personalities, suggesting that their development could play a key role in the early stages of speciation and adaptive radiation.

Introduction

The extent to which individual differences in behavior may play a role in mate choice, speciation and adaptive radiation is a fundamental question that stands at the interface between behavioral ecology and evolutionary biology. Differences among individuals form the substrate on which natural selection can operate, and individual differences in behavior specifically have been noted for a long time (Darwin 1859; Hinde 1959; Bryan and Larkin 1972; Kornfield et al. 1982; Werner and Sherry 1987; Wilson 1998). The more recent notion of animal personality refers to exactly that, consistent behavioral differences among individuals across time and/or contexts (Gosling 2001; Sih et al. 2004*a*), and comes with the statistical concepts and tools to address such differences within a unified quantitative framework (Nakagawa and Schielzeth 2010). Over the last two decades, extensive evidence has been gathered showing that animal personalities are widespread across the animal kingdom including marine invertebrates (Mather and Anderson 1993), arachnids (Pruitt et al. 2008), amphibians (Brodin et al. 2013), fish (Conrad et al. 2011), reptiles (Cote and Clobert 2007), birds (Groothuis and Carere 2005) and mammals (Found and St. Clair 2016). Animal personalities can involve a number of behavioral traits that correlate with each other, forming what has been referred to as behavioral syndromes (Sih et al. 2004*a*), as well as ecologically relevant traits such as feeding preferences or dispersal (Bolnick *et al.* 2003), implying that individuals of a population are not necessarily ecologically equivalent but can instead have their own specialized niche (Chase and Leibold 2003).

Personality traits and behavioral syndromes may also have evolutionary implications (Wilson 1998; Wolf and Weissing 2012; Ingley and Johnson 2014; Delarue et al. 2015). They can for example be differentially adaptive in environments differing in

predation pressure, with higher survival resulting from reduced activity under high predation (Kruuk and Gilchrist 1997). Personality traits may contribute to nonrandom mating if individuals tend to mate assortatively with respect to personality traits or behavioral syndromes, providing a link between the ecology of such traits and reproductive isolation (Carere *et al.* 2005; Schuett *et al.* 2011; Kralj-Fišer *et al.* 2013; but see Ariyomo and Watt 2013 & Laubu *et al.* 2017 for studies where such a link was not found). This link is particularly relevant in the context of speciation since it constitutes the cornerstone of the ecological hypothesis of speciation, whereby reproductive isolation results as a by-product of ecologically based divergent selection (Schluter 2001). Finally, animal personality may also play a role in adaptive radiation. For example, Werner and Sherry (1987) report a variety of specialized feeding behaviors in Cocos finches (*Pinaroloxias inornata*) that are consistent over time and space, independent of age, sex and morphology. The link between individual differences in behaviour and reproductive isolation was not investigated in this case, but the fact that this variation parallels the range of feeding specializations that characterizes the radiation of the closely related Darwin's finches in the Galápagos Archipelago suggests that the development of animal personalities within a species might form the basis of adaptive radiation (West-Eberhard 2003).

The hamlets (*Hypoplectrus* spp, Serranidae), a group of simultaneously hermaphroditic reef fishes from the wider Caribbean, provide a rare marine equivalent to the classic terrestrial and freshwater adaptive radiations such as Darwin's finches or East African cichlids. The hamlets have diversified into at least 17 species that are broadly sympatric (Aguilar-Perera and González-Salas 2010; Holt et al. 2010; Lobel 2011; Victor 2012; Tavera and Acero 2013) and differ almost exclusively in terms of color pattern

(Randall 1968; Whiteman et al. 2007; Holt et al. 2008). Hamlets can be observed spawning on a daily basis throughout the year during the hour preceding sunset. They spawn in pairs and trade eggs, alternating sex roles several times during a single spawning bout (Fischer 1980a). Fertilization is external, eggs and larvae are planktonic and there is no parental care (Fischer 1980a). Sympatric species spawn at the same time and often in close proximity, yet mating is strongly assortative, with > 98 % of pairings occurring between members of the same species (Fischer 1980b; Puebla et al. 2007, 2012; Barreto and McCartney 2008). Nevertheless, hybrid spawnings do occur at a low frequency (< 2 %) and there do not appear to be intrinsic post-zygotic barriers among species (Whiteman and Gage 2007). From a genetic perspective the hamlets encapsulate the entire continuum of genomic divergence, from no detectable divergence at mitochondrial and microsatellite markers up to well-diverged species in the Gulf of Mexico (McCartney et al. 2003; Holt et al. 2011; Puebla et al. 2012, 2014; Victor 2012; Tavera and Acero 2013). As such they provide a rare marine window into the process of speciation and adaptive radiation.

Hamlets are predators that feed on small invertebrates and fishes (Randall 1967; Whiteman et al. 2007). Randall and Randall (1960) and Thresher (1978) proposed that several hamlet species might be aggressive mimics (fig. 1 *A*). According to this hypothesis, the predatory hamlets (the mimics) gain an advantage in the approach and attack of prey by resembling other fishes from different families (the models) that are similarly-sized, more abundant and have distinct feeding strategies (e.g. mostly herbivorous, planktivorous or corallivorous). Thresher (1978) proposed that by providing a source of divergent selection on color pattern to match a variety of models, aggressive mimicry could have contributed to speciation and adaptive radiation in the hamlets.

Following up on this hypothesis from a behavioral perspective, Puebla *et al*. (2007) showed that in Bocas del Toro, Panama, the butter hamlet (*Hypoplectrus unicolor*, putative mimic) spends on average about 10 % of its time actively tracking the foureye butterflyfish (*Chaetodon capistratus*, putative model) but executes nearly 50 % of all predatory strikes during that time, which was interpreted as consistent with the aggressive mimicry hypothesis. Six years later, Robertson (2013) used the hamlets as a case study to propose an alternative view referred to as the 'social-trap hypothesis'. According to this hypothesis the resemblance between the model and mimic does not result from aggressive mimicry *per se* but from independent selection pressures such as predator avoidance, background matching or intraspecific social interactions, and the behavioral association between 'mimics' and 'models' is the consequence of an intrinsic tendency of the 'mimics' to socially respond to similar-looking fish. If such an interaction provides a benefit to the 'mimic' it is reinforced through learning, which could in turn set the stage for the evolution of mimicry (Robertson 2013).

Both the aggressive mimicry and social-trap hypotheses are relevant from a speciation and adaptive radiation perspective since both involve a source of natural selection driving resemblance between 'mimics' and 'models' and a benefit to the 'mimics' from associating with the 'models'. Nevertheless, for natural selection to operate and speciation to initiate, the tendency to associate with putative models needs to differ consistently among individuals, i.e. constitute a personality trait, and be linked to reproductive isolation. Here, we test these two hypotheses using behavioral observations of tagged individuals in a natural population of the butter hamlet, with aggressive mimicry behavior as the focal trait. We detail the natural history of this behavior and show that it differs consistently among individuals, forming two discrete behavioral types

that also differ with respect to foraging behavior and territoriality (‘aggressive mimics’ and ‘territorials’). In addition, we show that spawning tends to be assortative with respect to behavioral type in the study population, providing a link between aggressive mimicry, personality and reproductive isolation at the population level and a parallel with the large-scale patterns of speciation and adaptive radiation in *Hypoplectrus*.

Figure 1 *A*, Putative mimic-model pairs for aggressive mimicry in *Hypoplectrus*. Putative mimics (*Hypoplectrus* spp) and models are shown on the left and right column, respectively, and the red frame highlights the mimic-model pair considered in this study. From top to bottom: butter hamlet (*H. unicolor*) and foureye butterflyfish (*Chaetodon capistratus*), blue hamlet (*H. gemma*) and blue chromis (*Chromis cyanea*), yellowtail hamlet (*H. chlorurus*) and yellowtail damselfish (*Microspathodon chrysurus*), black hamlet (*H. nigricans*) and dusky damselfish (*Stegastes adustus*), 'tan hamlet' (*Hypoplectrus* sp) and threespot damselfish (*Stegastes planifrons*), shy hamlet (*H. guttavarius*) and rock beauty (*Holacanthus tricolour*), yellowbelly hamlet (*Hypoplectrus aberrans*) and cocoa damselfish (*Stegastes variabilis*, intermediate between juvenile and adult in this photograph). *B,* Other hamlet species for which no putative models for aggressive mimicry have been identified. Clockwise from top left to bottom right: barred hamlet (*H. puella*), indigo hamlet (*H. indigo*), masked hamlet (*H. providencianus*) and golden hamlet (*H. gummigutta*). Photographs from Paul Humann, with permission from Reef Fish Identification, New World Publications, © 2002, Paul Humann.

Methods

This study was conducted under the IACUC protocol 2013-0103-2016 and the *Autoridad de los Recursos Acuáticos de Panamá* research permit SE/A-61-15. Fieldwork was done in April-June 2014 and June-September 2015 on Punta Juan, an extensive reef located at the northern extremity of the Cristóbal island in the Bocas del Toro archipelago, Panama (9° 18.110' N 82° 17.660' W, see Guzman and Guevara (1999) for a detailed description of this reef).

Transect surveys

A total of 21 non-overlapping 4 x 100 m transects covering 8,400 m^2 of reef were conducted during the day on the study reef to test whether the putative model (*C. capistratus*) was more abundant than the putative mimic (*H. unicolor*) as implied by the aggressive mimicry hypothesis. Briefly, two scuba divers swam in parallel a few feet above the reef with each diver counting all fishes observed within two meters on each side of a 100 m transect tape, signaling any fish swimming across the transect tape to avoid counting the same individual twice. Seven transects were conducted on the shallow edge of the reef (10-15 ft), seven on the middle section of the reef slope (15-45 ft) and seven on the deep, patchy edge of the reef (45-55 ft).

Diurnal observations

In an effort to identify all the butter hamlets present on the reef, a total of 30 individuals were tagged with visible implant elastomer (Northwest Marine Technologies Inc.). Briefly, hamlets were collected with hook-and-line, photographed, fin-clipped, measured, tagged on the caudal fin using a combination of two to three colors and immediately released. The entire operation was realized *in situ* on scuba and took only a few minutes per fish. Individuals returned to their normal activities shortly after tagging. As far as we

could judge from both short- and long-term observations before and after tagging as well as negative controls in which individuals with particular natural markings were not tagged, tagging did not noticeably affect behavior or survival.

Following an acclimation period of at least two days after tagging, individuals were observed continuously and non-intrusively by two scuba divers during observation periods of 45 minutes, focusing on a single individual each time. These observations were performed on a subset of 19 tagged individuals that were repeatedly encountered in the study area after tagging. Each fish was observed on five to eleven different days, representing 156 observations for a total of 117 hours (fig. S1). Observations were conducted between 9:00 and 17:00 at depths ranging from 5 to 56 ft to assess behavior over a diversity of temporal contexts. Three individuals were surveyed over the two sampling periods in 2014 and 2015, spanning a period of 18 months. The behavioral data taken included *i.* time spent by *H. unicolor* tracking *C. capistratus*, *ii.* number of foraging bouts performed by *H. unicolor* when tracking *C. capistratus* and when not (referred to as while 'alone'), *iii.* time spent in territory if any, *iv.* number of aggressive chases performed towards other hamlets, and *v.* number of aggressive chases received from other hamlets and graysbies (*Cephalopholis cruentata*). 'Tracking' was defined as *H. unicolor* performing clear changes in speed and/or direction to actively stay within 30 cm of *C. capistratus* (see supplementary video sequence 1 in Puebla et al. (2007)). 'Foraging bouts' included predatory strikes, defined as clear accelerations directed towards a specific target (see supplementary video sequence 2 in Puebla et al. (2007) but also bites, nibbles or gulps that were performed without clear accelerations. 'Territories' were defined as actively defended reef areas of about 3 x 3 m containing a main coral head or sponge where an individual would be repeatedly encountered both during and outside of

observation periods. The aggressive chases performed and received were limited to the other hamlets and graysbies because these represented the vast majority of observations (aggressive chases by damselfishes were largely ignored by the hamlets and therefore not considered).

In addition, the target of all foraging bouts was recorded when identifiable. Such identifiable preys included masked or glass gobies (*Coryphopterus personatus*/*hyalinus*), juvenile slippery dicks (*Halichoeres bivittatus*), chalk basses (*Serranus tortugarum*) and small mysid aggregations in the water column. When the specific target was not identifiable, the target medium was recorded (sand, hard coral, soft coral, sponge). In this case it is not implied that hamlets prey on these media but rather that they target small prey on their surface. Additional relevant data including interactions with other fishes were also collected.

Spawning observations

The pairing and spawning behavior of the tagged individuals for which personality data was collected were carried out between June and September 2015 over a total of 42 dusk dives of 60 minutes each. Spawning observations focused on a 300 m stretch of reef where butter hamlets convened at dusk to spawn and included all the fish for which personality data was collected in 2015, except for the smallest individual that was never seen pairing (n = 11). Two divers followed tagged individuals on scuba and recorded pairings (defined as two individuals staying together and displaying to each other for at least 20 min), actual spawnings as well as courtship interactions (displays or aggressive chases) that did not necessarily result in pairings.

Repeatability

Repeatability, the proportion of behavioral variation that is due to differences between

individuals (Nakagawa and Schielzeth 2010), was estimated for each of the five above-mentioned traits to quantify the extent to which individual differences were consistent over time. Formally, repeatability is defined as $R = s^2_A / (s^2 + s^2_A)$, where s^2_A is the variance among individuals and s^2 the variance within individuals over time, and ranges between 0 and 1 (Nakagawa and Schielzeth 2010). Behaviors that show relatively low within-individual variance compared to among-individual variance are repeatable and may therefore be considered personality traits. Repeatabilities were estimated using generalized linear mixed models (GLMMs), with tag (individual identity) as random effect and observation periods as replicates. A Poisson distribution was considered for tracking time, time spent at home territory, chases performed and chases received since these data are counts (including time measurement that were treated as minute or second counts), and a binomial distribution was considered for the proportion of foraging bouts performed while tracking. These analyses were done in R (R Core Team 2016) using the *rptR* package (Stoffel et al. 2017) that considers mixed models fitted by the *glmer* functions (lme4 package, Bates et al. (2015)). 95% confidence intervals were estimated with 1000 bootstrap iterations and repeatabilities whose confidence intervals did not include zero were considered significant. Adjusted repeatabilities were also estimated to control for standard length and sampling year (2014, 2015 or both) by including these factors as fixed effects for all traits.

Behavioral syndromes

Pairwise correlations among the five behavioral traits recorded were explored with Spearman's rank correlation coefficients, considering mean trait values over observation periods for each individual. Statistical significance of each correlation coefficient was estimated using the *t* distribution and *p*-values were adjusted to correct for multiple

comparisons (Holm 1979). Mean values for each fish and the five behavioral traits were also visualized with non-metric dimensional scaling (NMDS) using Gower distances (Gower 1971). The number of dimensions was set to 2, resulting in a stress value of 0.078 that is considered to give a good ordination representation with low probability of misinterpretation (Clarke 1993). An agglomerative hierarchical clustering analysis (Kaufman and Rousseeuw 2005) was also applied to the pairwise Gower distance matrix to identify the optimal number of clusters in the dataset if present. Finally, permutational analysis of variance (PERMANOVA, Anderson (2001)) was applied to the distance matrix to test whether the clusters identified by the agglomerative hierarchical clustering analysis differed significantly (1000 permutations). These analyses were done with the v*egan* package in R (Oksanen et al. 2016).

Foraging behavior

The foraging bouts count data per category (i.e. directed towards masked/glass gobies, juvenile wrasses, chalk basses, mysids, sand, hard coral, soft coral or sponge, n = 8 categories) were too sparse to be analyzed on an individual basis as above, but they were used to test whether foraging behavior differs while tracking *C. capistratus* versus while alone. A Poisson-lognormal multivariate GLMM analysis designed for multivariate count data was applied using a Bayesian Markov chain Monte Carlo (MCMC) procedure with the MCMC.OTU package in R (Matz 2016). The model was fitted with non-informative over-dispersed priors for fixed effects, non-informative inverse-Wishart priors for the random effect (variance V = 1, degree of belief nu = 0) and weakly informative inverse-Wishart priors for the residual variance (V = 1, nu = number of food variables - 0.998, Hadfield, 2010). The model was run for 1 100 000 iterations, a thinning length of 1000 and a burn-in of 100 000. Food categories that were so sparsely represented that no

reliable parameter estimates could be obtained for them were discarded from further analysis. The foraging bout counts per food category and status (tracking/alone) for each observation period was used as the multivariate response variable. Each count was expressed relative to the total counts of bouts in each status of each observation period to account for the different amounts of time spent tracking and alone. Status was considered a fixed effect and fish identity a random effect, taking into account the fact that measures were repeated for each fish. Pairwise comparisons between 'alone' and 'tracking' for each food category were made based on their sampled posterior distributions. Their statistical significances were determines following Matz *et al.* (2013) and adjusted for multiple testing with false discovery rate correction (Benjamini and Hochberg 1995). A similar approach, detailed in the appendix part A, was used to test for differences in foraging behavior i. between the two behavioral clusters identified (see Results), ii. between the two behavioral clusters while tracking and iii. between the two behavioral clusters while alone.

Social network analysis

A social network analysis approach was adopted to analyze the pairing data and test whether mating tended to be assortative with respect to the identified behavioral clusters in the study population. A social network of the 144 pairing events spanning the 42 spawning observation periods was constructed using the *asnipe* (Farine 2016*a*) and *igraph* (Csardi and Nepusz 2006) packages in R. Since not all fish were sighted on every spawning dive, the half-weight association index (HWI, Cairns and Schwager (1987)) was used to estimate association strengths between pairs of individuals. The HWI ranges between 0 (never paired) and 1 (always paired) and accounts for potential sampling biases when individuals are only viewed on a fraction of all sampling events (Whitehead 2008).

All subsequent analyses were performed using the HWI.

In order to determine whether the network contains non-random structure, *i.e.* associations that are preferred or avoided compared to what is expected by chance, the coefficient of variation (CV) of the association index over all pairing observations was calculated and compared to the CV of 1000 null models built from randomized versions of the network using data-stream permutations (Bejder et al. 1998), which consist in randomly swapping pairings in the network. This approach accounts for the non-independence of social network data, for the presence/absence of individuals in the spawning area on each evening and for the number of observations made per individual by limiting permutations to the individuals that were present and displaying on each evening (even if not paired) and keeping the number of observations of each individual fixed (Farine and Whitehead 2015). A significantly higher CV of the real association indices compared to randomly permuted data would point to the occurrence of preferred or avoided pairings in the population (Farine and Whitehead 2015).

In order to test whether pairings tend to be assortative with respect to the identified behavioral clusters in the study population, the network's assortativity coefficient (Newman 2003) was calculated. This coefficient is a measure of the correlation between an individual's phenotype and that of its associates (mates in our case) that is commonly used to test for phenotypic structure in social networks (Farine and Whitehead 2015). The *assortnet* package (Farine 2016*b*) was used considering weighted networks (by the HWI in this case), which have been shown to be more robust than binary networks (Farine 2014). The previously identified behavioral clusters were used as categorical variables, generating an assortativity coefficient that can potentially range between -1 (fully disassortative) and 1 (fully assortative), with 0 corresponding to

random mating. Randomizations of the observed data were generated to test for assortative mating, using again data-stream permutations and resulting in 1000 randomized networks. The same approach was used to test whether pairing tended to be assortative with respect to size, with standard length used as a continuous variable in this case.

Individual-based model

The pairing observations indicated that individuals from the first behavioral cluster tended to meet at four well-defined 'rendezvous' sites on the reef for spawning as previously described in Puebla *et al*. (2012). In contrast, similarly to what was observed during the day, individuals from the second behavioral cluster kept swimming over the entire spawning area at the time of spawning. Since the randomizations described above do not take this spatial component into account, assortative mating was also tested against random expectations using a spatially explicit, individual-based model tailored to the empirical observations (detailed in the Appendix part B1). Briefly, the spawning area was represented as a 71 x 71 m grid, wrapped into a torus to avoid edge effects. This corresponds to an area of 5041 m^2, which is in line with the spawning area surveyed in the field. Four 'rendezvous' sites of 5 x 5 m were randomly placed in the grid and the presence or absence of eight (cluster 1, 'territorials') and three (cluster 2, 'aggressive mimics') individuals was defined according to empirical frequencies (i.e. the probability of finding each individual in the spawning area over the 42 evening dives). The 'territorial' individuals had a static behavior and occupied the 'rendezvous' sites only. Thus, assortative mating among these individuals is implied and not tested by the model; it is assortative mating among the 'aggressive mimics' specifically that is tested. The 'aggressive mimics' were randomly placed in the grid and moved to any of the eight

adjacent cells with a velocity of 30 cm s^{-1}, a value deduced from real swimming speeds in Serranidae (Fisher and Hogan 2007; Fulton 2007) and consistent with what was observed in the field. Each run consisted of 1080 time steps of 3.3 s each, corresponding to one hour total (the duration of the spawning observations). Mating occurred when two individuals not already paired were present in the same cell (at which point the 'aggressive mimics' would stop swimming) and lasted until the end of the simulation.

A total of 1000 simulations sets were performed, with 42 independent runs per set corresponding to the 42 observation periods. The position of the four mating sites was held constant over the 42 runs, with the presence/absence of individuals and the starting position of the 'aggressive mimics' varying for each run. The frequency of assortative versus disassortative pairings over the 1000 simulation sets was then compared to the empirical observations. Finally, a sensitivity analysis was conducted to evaluate the robustness of the results with respect to model parameters (detailed in the Appendix part B2). All simulations were performed in R.

Results

Transect surveys

A total of 21 transects covering 8,400 m^2 of reef provided a density estimate of 0.25 ± 0.05 individuals per 100 m^2 for *H. unicolor* on the study reef (mean ± s.e.) and 14 times higher densities for *C. capistratus* (3.5 ± 0.43 individuals 100 m^{-2}, mean ± s.e.). The barred (*H. puella*) and black (*H. nigricans*) hamlets were also about one order of magnitude more abundant than *H. unicolor*, with densities of 2.64 ± 0.21 and 1.77 ± 0.20, respectively (individuals 100 m^{-2}, mean ± s.e.). Two yellowbelly hamlets (*H. aberrans*) were also sighted within the transects, implying a density of 0.02 ± 0.01 individuals 100 m^{-2} of reef for this species (mean ± s.e.).

Diurnal observations

Butter hamlets were repeatedly observed tracking foureye butterflyfishes, for a total of 792 occurrences over the entire study. This was not a passive association but the result of an active behavior, with butter hamlets performing clear changes in direction and velocity to stay in close proximity to the foureye butterflyfish.

This behavior was also specific to the butter hamlet and foureye butterflyfish in particular. By and large, butter hamlets did not track other fishes and such associations were observed on only 25 occurrences (with slippery dicks, ocean surgeonfish (*Acanthurus bahianus*), threespot and dusky damselfish (*Stegastes planifrons* and *S. adustus*, respectively) and French grunts (*Haemulon flavolineatum*)). This was often the case when these fishes disturbed the sediment or small pieces of coral while foraging, and foraging bouts by butter hamlets were sometimes observed during these associations (5 occurrences), suggesting that they were opportunistically picking up prey dislodged by these fishes while foraging. The association with the foureye butterflyfish was of a clearly different nature, not restricted to cases when the sediment or small pieces of coral were disturbed. It was also of a different nature from the reported association between barred hamlets and striped parrotfishes (*Scarus iserti*), in which barred hamlets take advantage of the disturbance and distraction created by dense schools of tens to hundreds of parrotfish (Ogden and Buckman 1973; Robertson et al. 1976, not observed during the present study). The mean number of butterflyfishes tracked by butter hamlets was 1.6 (s.e. = 0.8), which did not create a strong disturbance on the reef. Interactions between butter hamlets and other hamlets present on the reef (black, barred and yellowbelly) were mostly aggressive during the day and butter hamlets were not observed tracking other butter hamlets or any other hamlet species. The other hamlet species were not the focus of

the behavioral observations presented here but it is noted that they were never observed tracking the foureye butterflyfish as the butter hamlet.

Repeatability

A total of 117 hours of continuous non-intrusive observation indicated that butter hamlets spent only 4.2 % of their time tracking *C. capistratus*, but did 22.4 % of all their foraging bouts during that time. Yet the amount of time spent tracking *C. capistratus* was significantly repeatable after adjusting for standard length and sampling year (R = 0.408, CI = 0.072 - 0.465, Table 1), implying that *H. unicolor* individuals differed consistently with respect to tracking behavior. The proportion of foraging bouts performed while tracking *C. capistratus* was also repeatable, but this was not the case anymore when adjusting for time spent tracking (R = 0.016, CI = 0.000 – 0.061), indicating that between-individual variation in proportion of foraging bouts performed while tracking *C. capistratus* can be explained by the variation in amount of time spent tracking *C. capistratus*. The amount of time spent at home territory was the most repeatable trait measured (R = 0.721, CI = 0.398 – 0.787 after correcting for size and sampling year, Table 1), pointing to marked individual differences in spatial use of the reef. The number of aggressive chases received was also repeatable but the number of aggressive chases performed towards other hamlets was not (Table 1).

Table 1. Repeatability estimates and 95 % confidence intervals for the five behavioral traits considered in this study.

Repeatability	**Tracking time** [95% CI]	**Prop. of foraging bouts while tracking** [95% CI]	**Time spent in territory** [95% CI]	**Chases performed** [95% CI]	**Chases received** [95% CI]
Unadjusted	**0.433** [0.133 - 0.540]	**0.292** [0.102 - 0.473]	**0.728** [0.524 - 0.808]	0.133 [0.000 - 0.294]	**0.499** [0.271 - 0.646]
Adjusted for size and sampling year	**0.408** [0.072 - 0.465]	**0.290** [0.078 - 0.438]	**0.721** [0.398 - 0.787]	0.059 [0.000 - 0.165]	**0.479** [0.149 - 0.584]

Behavioral syndromes

A NMDS plot integrating the five behavioral traits considered over the 117 hours of diurnal observation is presented in fig. 2. Hierarchical clustering analysis identified two clusters that match to the two groups apparent in the NMDS plot (fig. S2) and PERMANOVA indicated that these two clusters differ significantly (pseudo $F_{1,18} = 47.63$, $p < 0.001$). The same two clusters were identified when considering only the two traits linked to aggressive mimicry, i.e. time spent tracking *C. capistratus* and proportion of foraging bouts performed while tracking *C. capistratus* (fig. S3). The mean values of each behavioral trait for the two clusters are presented in Table 2. The most important difference between the two groups was the time spent in their territories, with 39.1 ± 1.8 versus 5.5 ± 3.3 minutes per observation period for clusters 1 and 2, respectively (mean ± s.e.). Another important difference was the time spent tracking *C. capistratus*, with 30.4 ± 7.1 versus 194.3 ± 15.2 seconds per observation period for clusters 1 and 2, respectively, as well as the proportion of foraging bouts performed while tracking *C. capistratus* (9.7 ± 0.1 versus 50.0 ± 0.1 for clusters 1 and 2, respectively, mean ± s.e.). The number of aggressive chases received was close to three times higher for cluster 2 but the number of aggressive chases performed towards other hamlets was similar between the two groups (within each other's standard errors).

In sum, two non-overlapping behavioral clusters were identified, with individuals in cluster 1 (referred to a 'territorials') spending relatively more time in their territories, less time tracking *C. capistratus* and receiving fewer aggressive chases from other hamlets and graysbies. Individuals in cluster 2 (referred to as 'aggressive mimics') spent relatively more time tracking *C. capistratus*, less time in their territories and received more aggressive chases from other hamlets and graysbies. Individuals of the two groups

differed clearly in terms of their diurnal behavior and differences could be extreme, with four ‘aggressive mimics’ not having a diurnal territory at all and three ‘territorials’ never observed tracking *C. capistratus.*

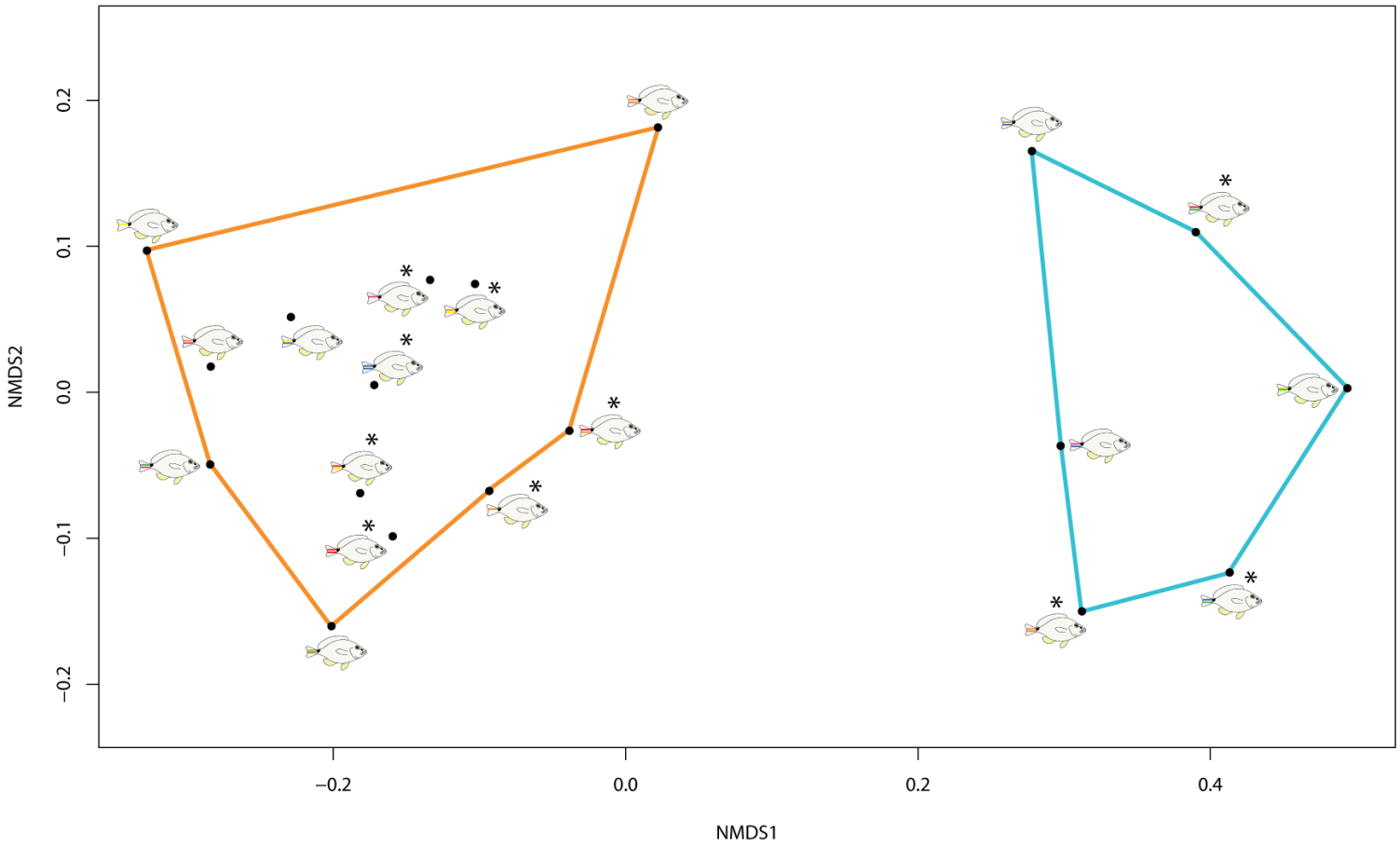

Figure 2. Non-metric multidimensional scaling (NMDS) summarizing the five behavioral traits listed in Table 1 for 19 butter hamlets (*Hypoplectrus unicolor*) from the Punta Juan reef, Bocas del Toro, Panama. Fish illustrations indicate the identity of each fish, represented by their unique tags on caudal fins. The two polygons delineate the two groups identified by the hierarchical clustering analysis (fig. S2), with orange corresponding to the 'territorials' (n = 13) and blue to the 'aggressive mimics' (n = 6). Fish marked with a star indicate individuals for which diurnal behavioral observations were made in 2015 and for which pairing data was collected as well (see fig. 4*A*).

Table 2. Mean values and standard errors of the five behavioral traits for ‘territorial’ (n = 13) and ‘aggressive mimic’ (n = 6) butter hamlets.

	Tracking time mean ± s.e. (sec)	**Foraging bouts while tracking mean ± s.e. (proportion)**	**Time spent in territory mean ± s.e. (min)**	**Chases performed mean ± s.e. (count)**	**Chases received mean ± s.e. (count)**
Cluster 1 ('territorials')	30.35 ± 7.08	9.71 ± 0.05	39.05 ± 1.80	1.12 ± 0.21	8.42 ± 1.63
Cluster 2 ('aggressive mimics')	194.29 ± 15.23	49.99 ± 0.11	5.48 ± 3.27	0.85 ± 0.27	22.11 ± 1.87

Correlations among the five recorded traits are presented in Table 3. The most strongly correlated pair of traits were time spent tracking *C. capistratus* and proportion of foraging bouts performed while tracking (ρ = 0.965, p < 0.001), indicating here again that the number of foraging bouts performed while tracking *C. capistratus* is strongly linked to the time spent tracking *C. capistratus*. The number of aggressive chases received from other hamlets and grasybies was also strongly correlated with the time spent at home territory (ρ = -0.849, p < 0.001), with individuals spending more time at home territories receiving fewer aggressive chases. The number of aggressive chases received was also positively correlated with time spent tracking *C. capistratus* (and proportion of foraging bouts performed while tracking *C. capistratus*) and the time spent at home territory negatively correlated with these two traits. All in all, these results are in line with the clustering analysis, indicating that time spent tracking, proportion of foraging bouts while tracking, territoriality and propensity to get chased by other hamlets and graysbies are strongly linked.

Table 3. Correlations (upper diagonal) and associated *p*-values (lower diagonal) among the five behavioral traits considered in this study.

	Tracking time	**Foraging while tracking**	**Time spent in territory**	**Chases performed**	**Chases received**
Tracking time	-	**0.965**	**-0.777**	-0.102	**0.788**
Foraging while tracking	**<0.0001**	-	**-0.753**	-0.098	**0.819**
Time spent in territory	**0.0006**	**0.0010**	-	0.190	**-0.849**
Chases performed	1.0000	1.0000	1.000	-	-0.026
Chases received	**0.0004**	**0.0001**	**<0.0001**	1.000	-

Bold: significant correlations at the 0.05 significance level.

Foraging behavior

A total of 1322 foraging bouts were observed over the entire study. The proportions of foraging bouts of each category performed while tracking *C. capistratus* versus while alone are presented in fig. 3. Of the eight categories analyzed, reliable parameter estimates could be obtained for six of them (all except soft coral and chalk basses). GLMM analysis indicated that foraging behavior differed while tracking versus while alone, with significantly ore foraging bouts towards masked/glass gobies (Bayesian z-score = 0.77, $p = 5.4.10^{-8}$) and significantly less towards mysids (Bayesian z-score = -0.51, $p = 0.0002$) while tracking *C. capistratus*. A similar result was obtained for the comparison between the two behavioral clusters (fig. S4). In this case the 'aggressive mimics' were found to attack significantly more masked/glass gobies than the 'territorials' (Bayesian z-score = -0.34, $p = 0.02$), but no differences were observed when tracking (fig. S5) or when alone (fig. S6), indicating that differences in foraging behavior between the two groups are due to differences in time spent tracking *C. capistratus.*

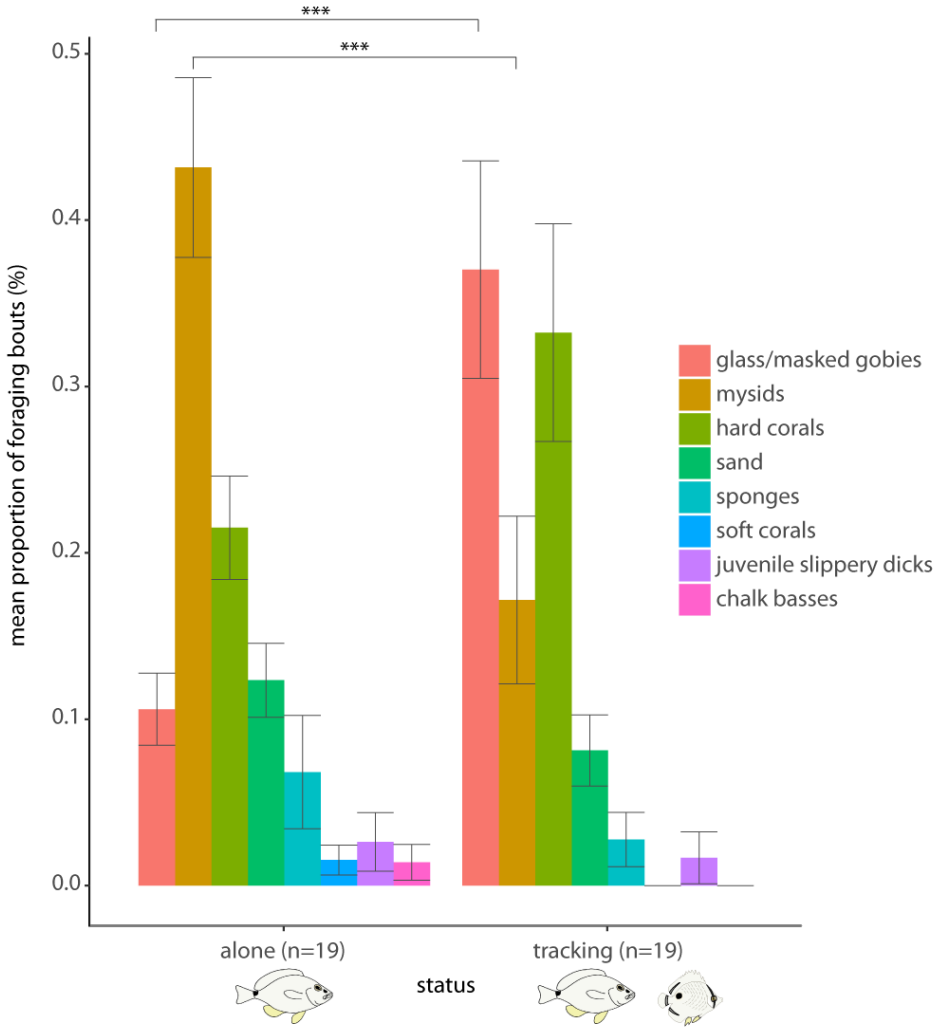

Figure 3. Proportion of foraging bouts performed by butter hamlets while alone versus while tracking the foureye butterflyfish on eight different food categories or media (note that in the latter case it is not implied that hamlets prey on these media but rather that they target small prey on their surface). Proportions were averaged for each fish first and then for each status (alone or tracking). *** significant differences at the 0.001 level, derived from multivariate GLMM analysis (see Methods for details).

Social network analysis

A total of 144 pairings (including 75 actual spawnings) were observed (fig. 4*A*). The overall number of pairings observed per individual ranged between 11 and 37 (mean = 26.2, s.e. = 2.4) and the number of partners between 1 and 5 (mean = 3.1, s.e. = 0.5). The 'territorials' tended to meet at four well-defined 'rendezvous' sites on the reef (different from their territories) for spawning as previously described in Puebla *et al*. (2012). In contrast, similarly to what was observed during the day, the 'aggressive mimics' used to swim over the entire spawning area at the time of spawning. The 'territorials' also tended to form stable pairs among them, with three pairs observed on 28, 29 and 37 of the 42 dusk dives.

The CV of the network association strength index (283.3) was significantly higher than the one derived from the 1000 randomized versions of the network ($p = 0.014$), pointing to non-random pairing in the population. The network's weighted-edge assortativity coefficient was positive ($r = 0.31$, s.e. = 0.16), indicating that butter hamlets tended to pair assortatively with respect to the two behavioral clusters, and the data-stream permutations indicated that this trend was significantly stronger than expected by chance ($p = 0.006$, fig. 4*B*). In contrast, pairing was not assortative with respect to size. In this case the weighted-edge assortativity coefficient was negative and non-significant ($r = -0.34$, s.e. = 0.29, $p = 0.067$).

Individual-based model

The results of the individual-based model are summarized in fig. 4*C* and *D* and detailed in the Appendix part B. Fig. 4*C* illustrates the movement of an aggressive mimic over the course of a simulation. Since the model assumes assortative mating among the 'territorials' by placing them at spawning 'rendezvous' sites only, it is the 'aggressive mimics' in particular that are considered

here. Only three of the 1000 replicate simulation sets provided a number of assortative matings among ‘aggressive mimics’ larger than the observed one, which therefore fell outside of the two-tail quantiles at the 5 % significance level (fig. 4*D*). This result was robust to the swimming speed and pattern of the butter hamlets, size and number of mating sites and shape of the spawning area (Appendix Part B).

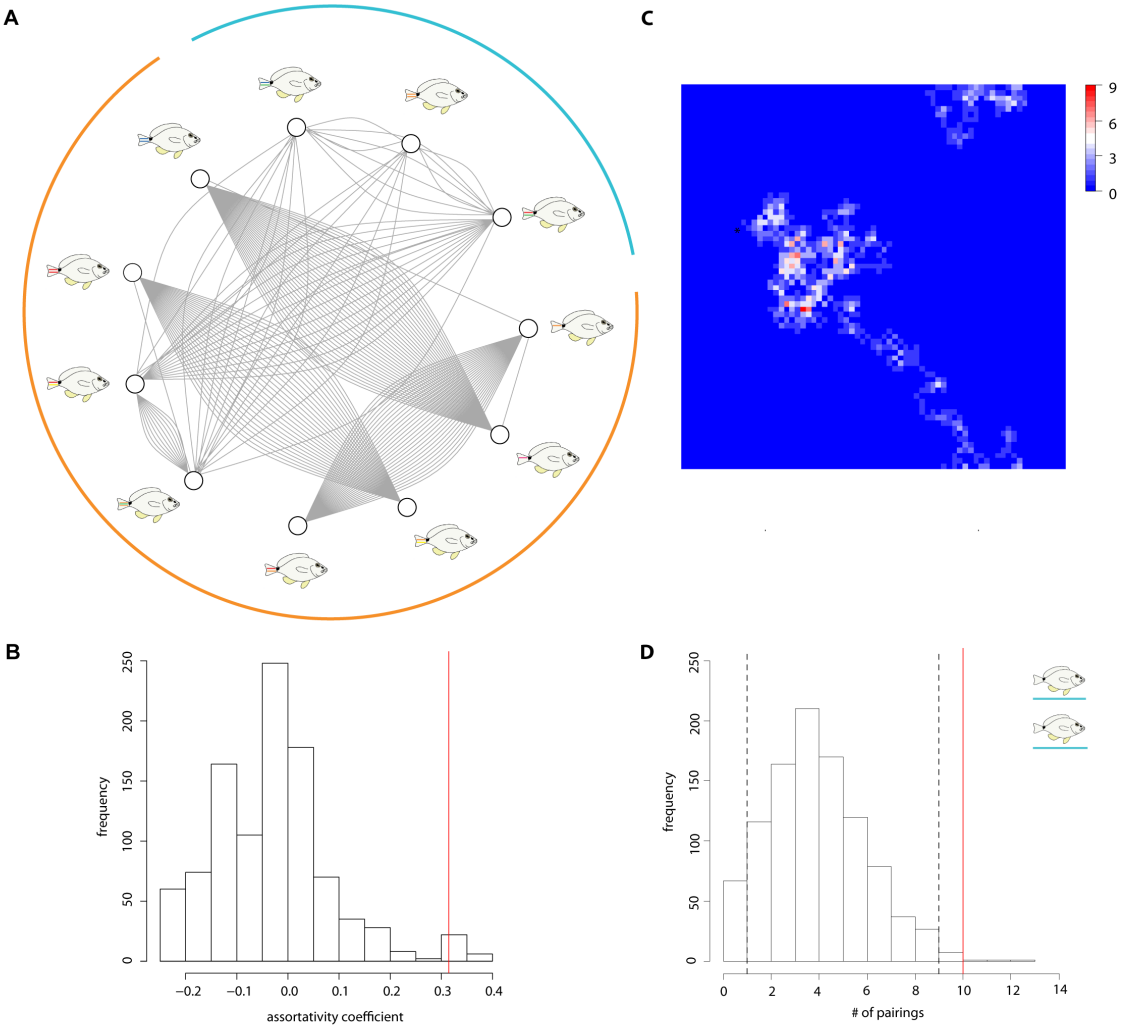

Figure 4 *A*, Pairing network representing 144 pairings sighted over 42 evening dives, with each line representing one pairing event. Colored arcs indicate behavioral cluster identity, with orange representing the 'territorials' and blue the 'aggressive mimics'. *B*, Frequency distribution of assortativity coefficients from 1000 randomized networks generated by data-stream permutations of the observed data (see Methods for details). The red line indicates the assortativity coefficient obtained from the observed data ($r = 0.31$). *C*, Illustration of the movement of an 'aggressive mimic' over the course of an individual-based simulation. Cell color is proportional to the frequency of passage, with warm colors indicating a larger number of transits (scale on the right). In this example the individual ended meeting and pairing with another 'aggressive mimic' (pairing point indicated with an asterisk). *D*, Frequency distribution of assortative matings among aggressive mimics as obtained from 1000 simulations. Thresholds of the two-tail quantiles at the 5 % significance level are delimited by dotted black lines and the number of assortative matings observed in the field by a solid red line.

Discussion

We used aggressive mimicry in the hamlets as a model system to explore the potential role played by animal personality in speciation and adaptive radiation. A total of 159 hours of non-intrusive observation in a natural population of butter hamlets from Panama indicated that aggressive mimicry behavior differs consistently among individuals and forms two discrete behavioral types that also differ with respect to foraging behavior and territoriality ('aggressive mimics' and 'territorials'). In addition, spawning observations indicate that mating tends to be assortative with respect to behavioral type in this population, providing a link between aggressive mimicry, animal personality and reproductive isolation.

Aggressive mimicry

Butter hamlets were repeatedly observed tracking foureye butterflyfishes, for a total of 792 occurrences over the entire study. Overall, they spent 4.2 % of their time tracking the foureye butterflyfish but did 22.4 % of all their foraging bouts during that time. These results are in line with Puebla *et al*. (2007), who reported that butter hamlets from the same area spent 10 % of their time tracking the foureye butterflyfish and did 50 % of all their predatory strikes during that time. The difference in the exact proportions between the two studies may be explained by the individual differences reported here (discussed below) as well as the fact that the current study focuses on the entire foraging spectrum (predatory strikes and bites) while Puebla et al. (2007) considered strikes only.

All in all, behavioral observations indicate *i*. that the association between the butter hamlet and foureye butterflyfish is the result of an active tracking behavior, *ii*. that this behavior is specific to the foureye butteflyfish in particular, *iii*. that it is associated with increased preying

activity and *iv*. that this increased preying activity is directed towards masked/glass gobies specifically. Considering in addition *v*. the general resemblance between the butter hamlet and the foureye butterflyfish (confusing to human observers at least when the two species are swimming side by side), *vi*. the fact that foureye butterflyfishes are 14 times more abundant than butter hamlets on the study reef and *vii*. that they feed almost exclusively on anthozoans (Birkeland and Neudecker 1981), we conclude that the tracking behavior of the butter hamlet is consistent with the aggressive mimicry hypothesis laid out by Randall and Randall (1960) and Thresher (1978), whereby the predatory hamlets (the mimics, butter hamlets in this case) gain an advantage in the approach and attack of prey (masked/glass gobies in this case) by resembling other fishes from different families (the models, foureye butteflyfishes in this case) that are similarly-sized, more abundant and have alternative feeding strategies (corallivores in this case). The fact that aggressive mimicry is associated with increased preying activity on masked/glass gobies specifically and not on invertebrates or even other fish prey (juvenile slippery dicks and chalk basses) suggests that this strategy is very specific in terms of its target prey. The behavior of the masked/glass gobies is also consistent with the aggressive mimicry hypothesis since they do not swim away from foureye butterflyfishes as they do from predatory fishes such as barred hamlets. Finally, the fact that butter hamlets spend only a small proportion of their time tracking the foureye butterflyfish is also consistent with the frequency-dependent nature of mimicry, since the masked/glass gobies might learn avoiding both the foureye butteflyfish and butter hamlets if the two were systematically associated.

Our behavioral observations do not necessarily invalidate the social-trap hypothesis (Robertson 2013) since it also involves an association between resembling ‘mimics’ and

'models'. Nevertheless, we note that butter hamlets are solitary, that they did not associate with other butter hamlets during the day and were never observed tracking other butter hamlets, and that the majority of diurnal interactions among them included aggressive chases (83 %, with no interactions for the remaining 17 % of encounters). Butter hamlets do therefore not appear to be socially attracted to similar looking fish as implied by the social-trap hypothesis. Considering the aggressive nature of the interactions between butter hamlets during the day, if anything they might be expected, on the contrary, to avoid visually similar fish in non-mating contexts. Nevertheless, the convergent evolution of color pattern put forward by the social-trap hypothesis might still be valid even if learned benefits from associating with 'models' do not originate from a tendency to associate with like specifically, but from a more general ability to explore new situations and learn from them as discussed below.

Personality and behavioral syndrome

Repeated observations of tagged butter hamlets indicate that aggressive mimicry behavior varies consistently among individuals, that this variation is not associated with body size and therefore not ontogenetic with an aggressive mimicry phase during development as in other reef fishes (Randall 2005), and that these individual differences in behavior can be maintained over up to a year and a half. Aggressive mimicry may therefore be considered a personality trait in the butter hamlet. Assessments of individual behavioral differences are often based on short-term controlled interactions as opposed to long-term observations of behavioral consistency in natural populations (but see Harrison *et al.* 2015; Found and St. Clair 2016; Villegas-Ríos *et al.* 2017), and this is particularly true in marine systems (Conrad et al. 2011) due notably to the logistical constraints imposed by the marine environment. Unusual interactions outside of natural habitats

often represent a biased snapshot of the complex social environment individuals are embedded in (Krause et al. 2010) and are not expected to elicit the same expression of behavioral variation that is expressed in the wild (Bell et al. 2009; Niemelä and Dingemanse 2014). This clearly applies to the current study, where the expression of aggressive mimicry relies on the presence of models, preys and extensive reef habitat.

An unexpected outcome of the behavioral observations is that individuals cluster into two discrete groups with respect to aggressive mimicry behavior, with a group of 'territorials' that spent on average 1.1 % of their time tracking the foureye butterflyfish and did 9.7 % of all their foraging bouts during that time, and a group of 'aggressive mimics' that spent on average 7.2 % of their time tracking the foureye butterflyfish and did 49.9 % of all their foraging bouts during that time. Since tracking behavior is associated with an increased frequency of predatory bouts directed towards glass/masked gobies in particular, the 'aggressive mimics' also preyed significantly more on this prey category. Another outcome of the behavioral observations is that aggressive mimicry behavior strongly correlates with territoriality, with 'territorials' being more territorial than 'aggressive mimics' (86.8 % versus 12.2 % of time spent at home territory on average, respectively). This difference is not simply the consequence that individuals cannot be both in their territories and tracking at the same time since the 'aggressive mimics' still spent 92.8 % of their time not tracking and the relatively high density of foureye butterflyfishes on the study reef implies that finding them does not require long search times. Thus, the 'aggressive mimics' could potentially spend a high proportion of their time in territories, but they instead keep swimming over the reef. One consequence of this difference in territoriality is that the 'aggressive mimics' received more aggressive chases from other hamlets and graysbies than the

'territorials' (22 versus 8 chases per 45 min observation period on average, respectively), most of which occurred when the 'aggressive mimics' crossed the territories of other hamlets or graysbies while swimming over the reef. It is to be noted, however, that territories did not appear to be limiting on the study reef. They were typically in the order of 10 m^2 and total hamlet density (all species confounded) on the study reef was estimated to 4.7 individuals per 100 m^2, indicating that about half of the reef was free of hamlet territories.

In sum, aggressive mimicry behavior in the butter hamlet varied consistently among individuals and defined two groups, with 'aggressive mimics' spending more time tracking the foureye butterflysfish and less time in their territories (if any), preying more on glass/masked gobies and receiving more aggressive chases from other hamlets and graysbies while swimming over the reef. We thus identified a co-varying suite of behaviors that were consistent across time and contexts, indicating that aggressive mimicry may be considered a behavioral syndrome (Sih et al. 2004*a*) in the butter hamlet. Such behavioral syndromes are not uncommon in animals (Sih et al. 2004*b*), but their ultimate causes are still an evolutionary puzzle even though a number of hypotheses focusing on either potential constraints (Sih et al. 2004*a*; Wolf and Weissing 2012; Dochtermann and Dingemanse 2013) or adaptive causes (Sih et al. 2004*b*; Wolf et al. 2007; Smith and Blumstein 2008; Bergmüller and Taborsky 2010) have been put forward. Here, the bimodal phenotype distribution suggests that disruptive selection might be the driving force favoring two alternative behavioral strategies with distinct fitness benefits (Bergmüller and Taborsky 2010). Pinpointing fitness benefits for each behavioral type falls outside the scope of this study, but the behavioral observations presented here suggest that the 'aggressive mimics' might benefit from higher preying success on glass/masked gobies while the 'territorials' might

be better protected from aggression and predation by spending more time in their territories. In addition, the frequency-dependent nature of mimicry has implications for the distribution of the two behavioral phenotypes. Any fitness benefit accruing to the 'aggressive mimics' would intrinsically depend on the frequencies of each behavioral type (Dall et al. 2004) since the occurrence of too many 'aggressive mimics' could lead to a decline in success of the aggressive mimicry strategy, through learning by the target prey (Cheney and Côté 2005; Cheney 2008).

Assortative pairing

Pairing observations indicated that individuals from the two behavioral clusters also behaved differently at the time of spawning, which extends the behavioral syndromes described above to the mating arena. Specifically, the 'territorials' met at specific 'rendezvous' points on the reef at the time of spawning as described by Puebla et al. (2012), while the 'aggressive mimics' kept swimming over the entire spawning area. Due to the low densities of butter hamlets on the study reef the number of individuals that could be observed spawning was relatively low, but they included all the individuals for which diurnal observations had been made in 2015 (except for the smallest one that was never observed pairing or spawning) and since the hamlets mate on a daily basis a total of 144 pairings could still be observed over the entire study. Both the data-stream permutations of the pairing network and the individual-based simulations indicated that pairing tended to be assortative with respect to behavioral type (but not size) in the study population. The pairing data and simulations suggest that this was driven by both the 'territorials', who tended to meet at specific 'rendezvous' sites for spawning and form stable pairs among themselves, and the 'aggressive mimics', who kept swimming over the entire spawning area but paired among

themselves more often than expected by chance when considering their spatial behavior in the individual-based simulations.

Assortative mating by personality type has been reported before and shown to result in higher fitness in several monogamous bird species with biparental care including great tits (Both et al. 2005), zebra finches (Schuett et al. 2011*a*), Stellar's jays (Gabriel and Black 2012) and eastern bluebirds (Harris and Siefferman 2014; Burtka and Grindstaff 2015) as well as in guppies (Ariyomo and Watt 2013), convict cichlids (Laubu et al. 2016) and the mound-building mouse (Rangassamy et al. 2015). In a few cases (great tits (Carere et al. 2005), zebra finches (Schuett et al. 2011*b*) and orb-weaving spiders (Kralj-Fišer et al. 2013)), it could be shown that assortative mating resulted from preferences for similar personality types specifically, indicating that personality traits can be sexually selected. It remains to be shown whether this is also the case in *H. unicolor*, but aggressive mimicry in the hamlets provides a link with speciation and adaptive radiation that is lacking in other systems.

Animal personality, speciation and adaptive radiation

Due to their shallow levels of genomic divergence and the possibility to observe spawning in the wild throughout the year, the hamlets provide a rare marine window into the early stages of speciation and adaptive radiation. Our data show that aggressive mimicry, a trait that has been proposed to play a key role in adaptive radiation in the hamlets, varies consistently among individuals within a population of the butter hamlet and that mating tends to be assortative with respect to this trait, providing not only a link between aggressive mimicry, personality and reproductive isolation at the population level but also a parallel with the large-scale patterns of speciation and adaptive radiation in *Hypoplectrus*. We do not mean to imply that butter hamlets

from the study population are necessarily in the process of diverging into two species, but the population-level patterns reported here may inform us on the processes of speciation and adaptive radiation in the whole genus.

Our behavioral observations indicate that aggressive mimicry is a facultative strategy in the butter hamlet, which is also true of the *Hypoplectrus* radiation at large since several hamlets do not have putative models for aggressive mimicry (fig. 1*B*). The behavior of the 'territorials' was essentially similar to the behavior of the barred hamlet, which is territorial, not an aggressive mimic (Puebla et al. 2007) and has been proposed to represent the ancestral form of the hamlet radiation (Thresher 1978; Puebla et al. 2008). Aggressive mimics and territorials are therefore recovered at both the population and radiation level. Not only was aggressive mimicry facultative at the population level, with some individuals consistently behaving as either 'aggressive mimics' or 'territorials', but also within individuals since the 'territorials' and 'aggressive mimics' were not exclusively so, with the 'territorials' still spending on average 1.1 % of their time tracking the foureye butterflyfish and the 'aggressive mimics' 12.2 % of their time in territories (which is why quotation marks are used throughout for 'territorials' and 'aggressive mimics'). Such a pattern whereby intraspecific variation parallels species differences has been found to be a key characteristic of other well-studied adaptive radiations such as East African cichlids (Kornfield et al. 1982) or Darwin's finches (Werner and Sherry 1987) and has been proposed to be indicative of diversification through developmental plasticity, whereby new forms emerge from plastic development in the ancestral phenotype (West-Eberhard 2003, 2005). This hypothesis resonates with the recent finding from a high-density genome scan that the only locus to be consistently diverged among black, barred and butter hamlets was close to *Hox* genes, which are known to

play a fundamental role in development (Puebla et al. 2014). In addition and importantly from a speciation perspective, aggressive mimics and territorials also tend to mate assortatively at both the population and radiation levels. Based on our observation of aggressive mimicry as a facultative and personality trait in the butter hamlet and a tendency to mate assortatively with respect to this trait, we propose the following scenario for speciation and adaptive radiation in *Hypoplectrus*.

Aggressive mimicry might have initially developed as a facultative strategy at the individual level. Some individuals might have specialized in this strategy and developed specific personalities as reported here. The different spatial use of the reef (higher mobility) implied by the aggressive mimicry strategy might have translated into a similar behavior at the time of spawning, as observed here in the butter hamlet, which could have contributed to initiate assortative mating. Aggressive mimicry and assortative mating may then have been reinforced through natural selection. This process could have been repeated in different populations considering not only the foureye butteflyfish but a variety of potential models as well (fig. 1*A*). Nevertheless, due to the fact that the 'territorial' strategy might also have benefits in terms of protection from aggression and predation and due to the frequency-dependent nature of aggressive mimicry, this strategy never evolved as obligate, which would explain why the hamlets are neither 'good mimics' (Robertson 2013) nor 'good species', with ongoing hybridization and low levels of genetic divergence.

The above scenario emphasizes the role played by behavior in speciation and adaptive radiation, but aggressive mimicry also implies a resemblance between models and mimics in terms of color pattern. In this respect it is important to stress that color pattern in the hamlets is

genetically determined (Domeier 1994) and is highly variable, not only between species but also both between and within populations of the same species (Thresher 1978; Aguilar-Perera 2004). This variation provides the substrate on which natural selection may operate, through not only aggressive mimicry but also the variety of processes such as predator avoidance, background matching or intraspecific social interactions put forward by the social-trap hypothesis that could also drive resemblance between 'models' and 'mimics' (Robertson 2013). Selection may even favor specific color patterns that do not resemble any other fish, which would explain why some hamlet species are not putative mimics. Such selective factors, if present, remain to be identified, but the color pattern of the barred and indigo hamlet (fig. 1*B*) have been proposed to be cryptic (Thresher 1978; Fischer 1980b). A detailed analysis of color pattern is beyond the scope of this study, but it is noted that the 'aggressive mimics' did not appear to better match the foureye butterflyfish than the 'territorials'. Behavioral differences were in contrast very clear, indicating that similarly to what was observed in Cocos finches (Werner and Sherry 1987), different ecologically relevant personalities can develop and be maintained in the absence of morphological differences.

Another question not addressed in this study is whether aggressive mimicry behavior has a genetic basis, but the fact that it varies among individuals suggests that it could possibly be a learned trait as proposed by the social-trap hypothesis (Robertson 2013). Learning has been found to play an important role in the development of individual behavioral differences in birds (reviewed in Giraldeau (1984)), insects, mammals, and fishes (Clark and Ehlinger 1987; Magurran 1993). As pointed out by West-Eberhard (2003, chapter 18), learning can be a potent source of adaptive evolution by recurrently generating advantageous morphology-behavior

combinations (resemblance-tracking in our case) that can become subject to selection and genetic accommodation, and this is where the social-trap and aggressive mimicry hypotheses may be reconciled. Exploratory behavior and learning are thought to have played a crucial role in adaptive radiation in Darwins's finches (Grant 1986; Werner and Sherry 1987). Very much like Darwin's finches, the hamlets are tame and exploratory, which is why it was relatively easy to approach them on scuba to capture them with hook-and-line for tagging and observe both their diurnal and spawning behaviors. Butter hamlets were generally curious towards the bait when capturing them with hook-and-line; they typically first explored it by swimming around it, projecting small water jets at it and slightly biting its edge before actually preying on it. When the first attempt to capture them failed, it was much more difficult to capture them afterwards. These observations may appear anecdotal but they illustrate the fact that hamlets are able to explore new situations and quickly learn from them.

This study highlights the complexity of individual behaviors that can develop and be maintained within populations, even in the absence of morphological differences, and therefore easily remain unnoticed unless specifically addressed through the repeated observation of individuals. More importantly, it illustrates the potential ecological and evolutionary implications that such individual behaviors can have and the importance of conducting detailed behavioral studies at the individual level to understand the evolution of phenotypic diversification.

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This section will be added after review to comply with the double blind policy of *The American Naturalist*.

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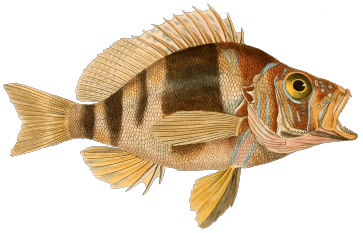
On the evolution of egg trading in simultaneous hermaphrodites



Chapter III: On the evolution of egg trading in simultaneous hermaphrodites (manuscript still in preparation)

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Abstract

Hermaphroditism is estimated to occur in about 65,000 animal species distribute over 24 phyla. Simultaneous hermaphroditism sets the stage for egg trading, whereby individuals trade each other's eggs for fertilization. Egg trading is of particular interest because it has been suggested to represent a rare case of cooperation among unrelated individuals and to stabilize simultaneous hermaphroditism, but it remains a rare phenomenon. While previous studies have addressed the stability of egg trading once established, how it may initially invade a population of non-trading simultaneous hermaphrodites remains an open question. Here, we address this question with an analytical model that considers egg production rate, egg senescence rate, encounter rates and costs of egg production on reproductive success in the male role in a population that may include three mating strategies: traders, providers, and withholders. The results indicate that a combination of sufficiently high costs and intermediate encounter rates allows traders to invade (and resist invasion from) both non-traders and cheaters. The model is calibrated with egg production rate, encounter rate, and cost estimates derived from new and extensive field observations of the butter hamlet (*Hypoplectrus unicolor*), an egg trading fish from the wider Caribbean. This case study is leveraged to make an explicit link between theory and biology, and to address additional aspects of the biology of egg trading that may explain why this phenomenon is not more widespread.

Introduction

In plants, hermaphroditism is referred to as dioecy and constitutes the norm, with about 6% of species only having separate sexes (Renner and Ricklefs 1995). The apparent absence of hermaphroditism in insects and its rarity in vertebrates, where it is restricted to about 1-2% of fishes (Pauly 2007; Avise 2011), might suggest at first sight that it constitutes an evolutionary singularity in animals. Yet a survey across the animal kingdom indicates that hermaphroditism occurs in 24 out of 34 animal phyla (70%) and is common to dominant in 14 phyla including sponges, corals, jellyfishes, flatworms, mollusks, ascidians and annelids (Jarne and Auld 2006). All in all, hermaphroditism is estimated to occur in about 65,000 animal species, which represents a third of animal species when insects are excluded (Jarne and Auld 2006).

Hermaphroditism may be sequential, with individuals being female or male at different times of their life cycle, or simultaneous, with individuals being both female and male at the same time. Simultaneous hermaphroditism sets the stage for self-fertilization, which is commonly observed at various degrees in both plants (Goodwillie et al. 2005) and animals (Jarne and Auld 2006). Yet simultaneous hermaphroditism also opens the door to gamete trading, whereby individuals reciprocally trade each other's gametes for fertilization (Leonard and Lukowiak 1984). Gamete trading is a form of reciprocity, variously referred to in the literature as delayed (Fischer 1988), conditional (Michiels and Streng 1998), serial, simultaneous, alternating (Anthes et al. 2006), cooperative (Crowley and Hart 2007), social (Crowley and Hart 2007), iterative (Crowley and Hart 2007) or direct (Henshaw et al. 2014)

reciprocity. It comes in two flavors, sperm trading and egg trading, both of which appear to be rare among simultaneous hermaphrodites.

Sperm trading is restricted to species with internal fertilization, and has been reported in sea slugs (Leonard and Lukowiak 1984; Anthes et al. 2005) and flatworms (Michiels and Streng 1998; Vreys and Michiels 1998). It has been proposed to be driven by the fact that females can control internal fertilization in some simultaneously hermaphroditic species (Leonard and Lukowiak 1984). Egg trading evolved independently in dorvilleid polychaetes in the genus *Ophryotrocha* (Sella 1985; Sella et al. 1997; Sella and Ramella 1999; Sella and Lorenzi 2000) and in Serraninae fishes (Fischer 1980*a*, 1984; Pressley 1981; Petersen 1995; Oliver 1997). Although egg trading has been interpreted in terms of control of fertilization by males (Leonard and Lukowiak 1984) and sexual signaling (Landolfa 2002), its most widespread interpretation rests on the extension of Bateman's principle (Bateman 1948) to simultaneous hermaphrodites (Charnov 1979). The idea is that since eggs are more energetically costly to produce than sperm, reproductive success is expected to be limited by access to eggs specifically. Mating in the male role should therefore be preferred, which creates a conflict between the two members of a mating pair. Egg trading provides one way to resolve this conflict by alternating sex roles and reciprocally fertilizing each other's eggs. In this case, reproductive success in the male role is limited by the release of eggs, and specific aspects of the biology of egg traders such as egg parceling or synchronized spawning may limit opportunities for ‘cheating', i.e. spawning in the male role predominantly or exclusively (Fischer 1980*a*).

Axelrod and Hamilton (1981) proposed that egg trading might constitute a case of cooperation among unrelated individuals corresponding to the tit for tat strategy in the Iterated Prisoner's Dilemma game (IPD), an idea extended by Fischer (1988). These two studies have been instrumental in drawing attention towards egg trading and stimulating further theoretical and empirical work, but the hypothesis itself was criticized on several grounds, some of which already acknowledged by Fischer (1988). These include specific assumptions of the IPD such as equivalent players, simultaneous decisions, or symmetric payoffs that are independent of prior behavior (Friedman and Hammerstein 1991; Connor 1992; Petersen 2006), specific aspects of the biology of egg trading such as search and display that are not considered under this framework (Friedman and Hammerstein 1991), the fact that the female component of fitness gains was omitted from the payoff matrix in Fischer's implementation of the IPD (Crowley and Hart 2007), and last but not least the fact that egg traders have the possibility to terminate the game at any time, i.e. leave the pair to seek a new partner (Friedman and Hammerstein 1991; Connor 1992; Crowley and Hart 2007). Alternative models (Friedman and Hammerstein 1991; Connor 1992; Crowley and Hart 2007) have led to a betted understanding of the dynamics and stability of egg trading, and to reconsidering its nature as a case of either non-cooperative equilibrium behavior (Friedman and Hammerstein 1991), pseudo-reciprocity (Connor 1992), or byproduct mutualism (Crowley and Hart 2007).

While the above-mentioned models addressed the dynamics and stability of egg trading once already evolved, how it may initially invade a population of non-trading simultaneous hermaphrodites is another question that turns out to be more problematic.

Axelrod and Hamilton (1981) speculated that egg trading might have evolved through a low-density phase that would have favored self-fertilization and inbreeding, which would have in turn allowed kin selection to operate, but this hypothesis was discarded on the ground that many egg traders do not (and might not have the physiological ability to) self-fertilize (Fischer 1981, 1988). More recently, Henshaw et al. (2014) provided a combination of analytical and simulation models that constitutes the first attempt to explicitly address the evolution of egg trading. Their analytical model considers encounter rates in a population that includes non-traders (who provide eggs at every mating opportunity, referred here as 'providers') and traders (who provide eggs only if their partner also does so). The results show that egg trading is under positive frequency-dependent selection in this framework, implying that it can go to fixation once a given proportion of the population is composed of egg traders, but leaving it open how it may initially arise in the first place. Here, we take a similar approach but extend it by adding two fundamental aspects of Bateman's principle; the costs of egg production on reproductive success as a male and the possible occurrence of withholders (`cheaters' who never provide eggs and only mate in the male role). We also add a rate at which eggs become non-viable if not fertilized. We show that these additions generate complex dynamics that allow traders to invade (and resist invasion from) both non-traders and cheaters when costs of egg production are sufficiently high and encounter rates intermediate.

Our approach is meant to capture the most relevant parameters for the evolution of egg trading as opposed to realistically model any egg trading species in particular. Nonetheless, in an effort to link the model to biology, an attempt is made to calibrate it with egg

production andsenescence rates, encounter rate and cost estimates derived from long-term field observations of the butter hamlet (*Hypoplectrus unicolor*). The hamlets are a group of simultaneously hermaphroditic reef fishes from the wider Caribbean that have played a key role in the study of egg trading, which was described for the first time by Fischer (1980) in the black hamlet (*Hypoplectrus nigricans*). Fischer's detailed account served as a reference for the description of this phenomenon in other species (Pressley 1981; Fischer 1984; Sella 1985; Petersen 1995; Oliver 1997; Sella et al. 1997; Sella and Ramella 1999; Sella and Lorenzi 2000), the development of egg-trading models (Axelrod and Hamilton 1981; Friedman and Hammerstein 1991; Connor 1992) and the interpretation of egg trading in general (Leonard and Lukowiak 1984; Landolfa 2002).

Hamlets spawn on a daily basis throughout the year during the last two hours before sunset (Fischer 1980*a*). They meet in a specific area of the reef for spawning, which is distinct from their diurnal territories. The major courtship display (head snap) is associated with spawning in the female role specifically and there is no display associated with spawning in the male role (Fischer 1980*a*). Courtship, aggressive, pairing and spawning behaviors are all clear and conspicuous, which provides the opportunity to estimate the main parameters of the model. Nevertheless, this requires long-term observations of tagged individuals, which are not available in the literature and reported here for the first time. This case study is also leveraged to address specific aspects of the biology of egg trading that are not included in the model and may explain why this phenomenon is not more widespread.

Materials and Methods

Mathematical model

Parameters

Our model builds on Henshaw et al. (2014) extending it in a number of directions. We posit a large, well-mixed population of simultaneous hermaphrodites. At any point in time, each individual in the population either is (*e*) or is not (*o*) carrying a batch of eggs. Individuals without eggs produce a new batch of eggs at a rate normalized to 1 (egg production rate), so that all following rates are measured relative to the rate of egg production. Potential mates are encountered at rate $m > 0$ if the focal individual carries eggs, or at a discounted rate λm, where $0<\lambda<1$, if the focal individual does not carry eggs. Equivalently, an individual not carrying eggs is not available for encounters with probability λ; λ hence captures the costs of egg production on reproductive success in the male role (an individual busy producing eggs cannot be available all the time as a potential partner in the male role). $\lambda=1$ implies no costs (individuals who do not carry eggs can always mate in the male role) and $\lambda=0$ implies maximal costs (mating in the male role is not possible when individuals do not carry eggs). Eggs become non-viable at rate $\rho \geq 0$. Finally, we assume a probability q of detecting actors not releasing eggs and of "punishing" them by not releasing eggs, where $0 < q < 1$ (applies only to traders detecting withholders, see below for strategies).

Strategies

There are three possible strategies: traders (T), withholders (H), and providers (P). All three strategies mate in the male role (*i.e.* fertilize eggs offered to them) whenever possible, but differ on the conditions under which they offer their eggs to partners for fertilization. Our traders behave like the traders in Henshaw et al. (2014): they offer their eggs only to partners carrying eggs (and hence, who can reciprocate). Withholders produce and carry eggs but never release those eggs to partners, essentially only reproducing through the male role. The only strategic function of their eggs is to elicit egg release from traders. Recall that traders can detect withholders with probability $0 < q < 1$ and "punish" them by not releasing eggs. Finally, providers correspond to the "non-traders" in Henshaw et al. (2014): they offer their eggs to any partner (either carrying or not carrying eggs). The model in Henshaw et al. (2014) is recovered from our more general model by: *i*. allowing only for providers and traders, *ii.* assuming egg production has no cost on male reproductive success (by setting $\lambda = 1$), and *iii.* ignoring egg senescence (by setting $\rho = 0$).

Proportions of strategies and of egg carriers

Let x, y, and z denote the respective proportions of traders, withholders, and providers in the population, satisfying:

$$x + y + z = 1, \; x \geq 0; y \geq 0; z \geq 0, \quad (1)$$

and let Δ denote the simplex of population shares (x, y, z) of the three strategies satisfying the conditions in (10). A simplex represents the set of all points whose coordinates are not

negative and add up to one. The corner points of the simplex (vertices) indicate populations that consist of only one type and will be labelled accordingly as P (indicating a population consisting of only providers), T (indicating a population consisting of only traders), and H (indicating a population consisting of only withholders). Along the edges of the simplex, only two strategies coexist and one has gone extinct, and in the center, all strategies can coexist. Within each strategy, individuals can either be carrying eggs or not carrying eggs. Let x_e, y_e, and z_e denote the proportions (relative to the overall population size) of, respectively, traders carrying eggs, withholders carrying eggs, and providers carrying eggs, with the corresponding proportions of individuals not carrying eggs then given by:

$$x_o = x - x_e, \quad (2a)$$
$$y_o = y - y_e, \quad (2b)$$
$$z_o = z - z_e. \quad (2c)$$

To abbreviate formulas, it will sometimes be convenient to use e and o to denote the population fractions carrying eggs, and respectively not carrying eggs:

$$e = ze + xe + ye, \quad (3a)$$
$$o = z_o + x_o + y_o. \quad (3b)$$

Evolutionary dynamics

We assume a haploid genetic system with a single locus (*i.e.* each individual's reproductive strategy is determined by a single gene, inherited from the mother or the father with equal probability). Moreover, we assume a separation of time scales, so that demographic variables (proportions of individuals carrying and not carrying eggs within each strategy) equilibrate much faster than evolutionary variables (proportions of individuals

implementing each strategy). To model the evolutionary dynamics, we make use of the replicator dynamics with total (expected) fitness, expressed according the following differential equations:

$$\dot{x} = x\,(w_{\mathrm{T}} - \bar{w})\,,\ \ \dot{y} = y\,(w_{\mathrm{H}} - \bar{w})\,,\ \ \dot{z} = z\,(w_{\mathrm{P}} - \bar{w}) \quad (17)$$

where the dots denote time derivatives. x, y, and z are, respectively, the proportions of providers, traders, and withholders in the population (which satisfy $x + y + z = 1$), and can vary within the simplex Δ. w_{T} , w_{H} , and w_{P} are the fitnesses, defined as the expected number of offspring produced via reproduction in both gender roles of each strategy. $\overline{w}$ is the average fitness in the population, where $\overline{w} = Pw_P + Tw_T + Ww_H$.

Following a similar methodology as the one followed by Henshaw et al. (2014) to analyze their model, we find that (up to a linear transformation that does not change our results) the fitnesses of the three strategies can be written as:

$$w_{\mathrm{T}} = \frac{x_e}{x}\left[2x_e + (1-q)y_e + (2-\lambda)z_e\right], \quad (18a)$$

$$w_{\mathrm{H}} = \frac{y_e}{y}\left[(1-q)x_e + (1-\lambda)z_e\right], \quad (18b)$$

$$w_{\mathrm{P}} = \frac{z_e}{z}\left[\lambda + (2-\lambda)x_e + (1-\lambda)y_e + 2(1-\lambda)z_e\right]. \quad (18c)$$

See appendix S1 for more details on the model.

Empirical calibration

Fieldwork

Fieldwork was realized between July 2013 and June 2014 off Punta Caracol (9 21'58"N 82 17'29"W) in Bocas del Toro, Panama, under the IACUC protocol 2013-0301-2016 and the Autoridad de los Recursos Acuaticos de Panama research permits number 13 and 23. The study reef is approximatively 300 m long and 85 m wide, representing an area of 25 500 m^2. It is surrounded by a deep sandy area, which prevents hamlets from swimming to or away from the reef. Nine hamlet species have been observed on this reef that range from rare (1-3 individuals) to abundant (hundreds of individuals, Puebla et al. (2011)). The butter hamlet was targeted for this study due to its intermediate abundance, which provides the opportunity to tag and observe all individuals from this species on that reef. Individuals were collected with hook-and-line, photographed, fin-clipped, measured, tagged with visible implant elastomer in the caudal fin (Northwest Marine Technologies Inc., Fig. 1) and immediately released. The entire operation was realized in situ on scuba and took only a few minutes per fish. As far as we could judge from observations before and after tagging as well as negative controls in which individuals with particular natural markings were not tagged, tagging did not noticeably affect behavior or survival.

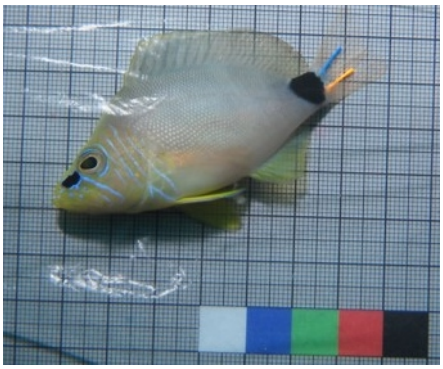

Figure 1 Picture of a tagged butter hamlet (with blue and orange).

Behavioral observations were initiated on August 15th 2013, at which point all butter hamlets found on the reef (n=19) were tagged, and repeated several times per week until June 16th 2014 for a total of 101 days. Observations were made by two scuba divers during the last two hours before sunset. They were mostly focused on the middle reef slope (mean depth 25 feet) where butter hamlets convened to spawn, but other parts of the reef were regularly visited as well to ensure that spawning interactions outside this area were not missed. The data recorded consisted of the timed sequence of all the individuals and spawning interactions observed during the dive including displays (as described by Fischer (1980)), aggressive chases, pairings (defined as two individuals staying together and displaying to each other for 20 minutes or more) and actual spawnings. Observations were not pair-centered as in Fischer (1980), i.e. they do not include the complete sequence of displays and spawnings for each pair but are instead meant to capture the spawning activity of the entire butter hamlet population on the study reef.

Parameter estimation

The fact that hamlets convene at a given time in a specific area for spawning and have clear displaying, pairing, and spawning behaviors provides an opportunity to estimate the main parameters (egg production rate, costs of egg production in terms of mating in the male role (λ), and mean encounter rate (m)) of the model from behavioral observations.

Hamlets produce new eggs on a daily basis and eggs that are not fertilized on the day they are produced become inviable (Fischer, 1981). Yet this does not necessarily imply that

hamlets produce new eggs on every single day of their sexually mature life and Fischer (1980) suspected that they might rest on some days, which has implications in terms of both egg production rate and costs of egg production. Egg production rate was estimated as the mean proportion of days on which individuals were assumed to carry eggs (observed displaying, pairing, or spawning in the spawning area) counting since the day they were observed displaying for the first time to ensure that only sexually mature individuals are considered:

$$\text{Egg production rate} = \frac{\sum_{i=1}^{Nsex} \frac{Number\ of\ days\ displaying\ and|or\ pairing}{Duration\ of\ sexually\ active\ life\ in\ days}}{Nsex}$$

where *Nsex* is the total number of sexually active fish in our observations.

This assumes that individuals who display produced new eggs on that day and that individuals who do not show up in the spawning area (or show up but do not display) did not produce new eggs, which is consistent with the fact that the major display in the hamlets is associated with spawning in the female role specifically and supported by both observational and experimental evidence (Fischer 1980*a*, 1981).

The costs of egg production in terms of mating in the male role (λ) were estimated as the mean proportion of individuals that are assumed to not have eggs but still the possibility to mate in the male role (*i.e.* that show up in the spawning area but do not display) among all the individuals that are assumed to not have eggs (*i.e.* that either show up in the spawning area but do not display or do not show up at all):

$$\lambda = \frac{\sum_{i=1}^{D} \frac{Number\ of\ fish\ present\ on\ the\ spawning\ area\ but\ not\ displaying}{Total\ number\ of\ fish\ assumed\ to\ not\ cary\ eggs}}{D}$$

where *D i*s the total number of sampling days (101 days in our observations).

This is consistent with the model, with $\lambda = 0$ corresponding to a situation where all the individuals who do not have eggs are not in the spawning area and can therefore not mate in the male role (maximal costs), and $\lambda = 1$ to a situation where all individuals are always present in the spawning area regardless of whether or not they have eggs and can therefore always mate in the male role (minimal costs).

Since the encounter rate (*m*) in the model applies to individuals that carry eggs specifically, only sexually active individuals (*i.e.* that show up in the spawning area and display) were considered for the estimation of this parameter. Encounter rate was estimated as the mean maximum number of encounters that each sexually active individual could have each evening by taking the mean of the total number of sexually active fish available in the spawning area from the point of view of each fish. It was estimated as:

$$m = \frac{\sum_{i=1}^{D}(Nfish-1)}{D}$$

where *Nfish* indicates the total number of sexually active fish present in the spawning area carrying eggs on each sampling day and *D* represents the total number of sampling days (D = 101 days). *m* was then rescaled to an egg production rate of 1 as assumed by the model for mathematical simplicity.

Results

Mathematical model

We proceed in four steps, which are detailed in Appendix S2. First, we obtain convenient expressions for the pairwise comparison of the renormalized fitnesses, which provide the basis for the subsequent steps (Appendix S2.1). Second, we show that the replicator dynamics has no interior rest point (Appendix S2.2), that is, there are no conditions where the three strategies can coexist. Third, we identify how the number, location, and stability of the rest points on the edges of the simplex Δ depend on the parameters of the model. Fourth, we look at how the parameter space is divided into different regions, each characterized by different dynamical portraits. Taken all together, these results provide us with a complete qualitative picture of the evolutionary dynamics underlying the evolution of egg trading.

Rest points and dynamics on the edges of the simplex

Our analyses identified that although no rest point could be identified within the simplex, one rest point could be identified along each edge of the simplex Δ, depending on the parameters of the model (see Appendix S2.3 for details of dynamics on the edges). The rest point (named *R*) along the Trader-Provider (TP) edge, when it exists, is an unstable equilibrium. R is unstable as it is repelling from both vertices T and P, but is attracting for neighboring points in the interior of the simplex. Hence, R is a source point. Similarly, the rest point (named *S*) along the Provider-Withholder (PH) edge, when it exists, is an unstable equilibrium. S is unstable as it is attracting from both vertices H and P, but is it repelling for neighboring

points in the interior of the simplex. The rest point along the Trader-Withholder (TH) edge (named *Q*), when it exists, is a stable equilibrium. Q is stable because it is attracting from all directions: from the vertices T and H, and from neighboring points in the interior of the simplex. Hence, Q is a sink (see Appendix S2.4 for details on the stability of the rest points).

Dynamical portraits

Our analytical model identified five qualitatively different regions within the simplex, all leading to different equilibria, depending on critical values of λ as a functions of *m*, on which the existence of the non-trivial points depend. These critical values are $\bar{\lambda}$, λ_*, and λ^*, defined as:

$$\bar{\lambda}(m) = \frac{m(1-q)^2 - (1-q^2)(1+\rho)}{(1+q)(1+\rho)(1+q+2\rho)},$$
$$\lambda_*(m) = \frac{m-(1+\rho)}{\rho(1+\rho)+m(2+\rho)},$$
$$\lambda^*(m) = \frac{m-(1+\rho)}{(1+\rho)(1+2\rho)}.$$

These regions exist for any given value of ρ (rate at which eggs become non-viable, where $\rho \geq 0$) and of *q* (probability of traders to detect cheaters, where $0 < q < 1$).

In all regions, H is always a source, and can never be evolutionarily stable. When they exist, Q is a sink (attracting for all points), R is a source (repelling for points along the TP-edge, attracting for interior points), and S is a saddle (attracting for points along the HP-edge, repelling for interior points). Each region is determined by different dynamical portraits (Fig. 2a):

1.region *i* (blue in Fig. 2a): If $\lambda \geq \lambda^*$, then there is no rest point on the edges, T is a

saddle (attracting from H and repelling from P), and P is a sink. P is the only evolutionarily stable outcome.

2. region *ii* (yellow in Fig. 2a) : if max ($\lambda_*, \bar{\lambda}$) < $\lambda \leq \lambda^*$, then R exists along the TP-edge, T is a sink, and P is a sink. T and P can both be evolutionarily stable.

3. region *iii* (green in Fig. 2a): If $\lambda_* < \lambda < \bar{\lambda}$, then R exists and Q exist, T is a saddle (attracting from P and repelling from H), and P is a sink. Both P and R are evolutionarily stable outcomes.

4. region *iv* (red in Fig. 2a): If $\lambda <$ min ($\bar{\lambda}, \lambda_*$) then S and Q exist, T is a saddle (attracting from P, repelling from H), and P is a source. Q is the only evolutionarily stable outcome.

5. region *v* (purple in Fig. 2a): If $\bar{\lambda} < \lambda < \lambda^*$, then S exists, T is a sink and P is a source. T is the only evolutionarily stable.

In sum, region *v* characterizes the only region where egg trading is the *only* evolutionarily stable strategy to evolve and resist invasion from providers or withholders. In zone *i*, egg trading goes to extinction as providers are the only stable strategy. In zone *ii*, egg trading may become evolutionary stable or extinct, depending on initial frequencies of each mating strategy. In zone *iv,* traders have to coexist with withholders. And in zone *iii*, traders either coexist with withholders or become extinct, depending on initial frequencies of each mating strategy.

Dynamical regions

Figure 2b shows how the five regions fit in the parameter space defined by λ and *m,* (in this case with egg production = 1, $\rho = 1$ and q = 0.5). See appendix S2.5 for more details.

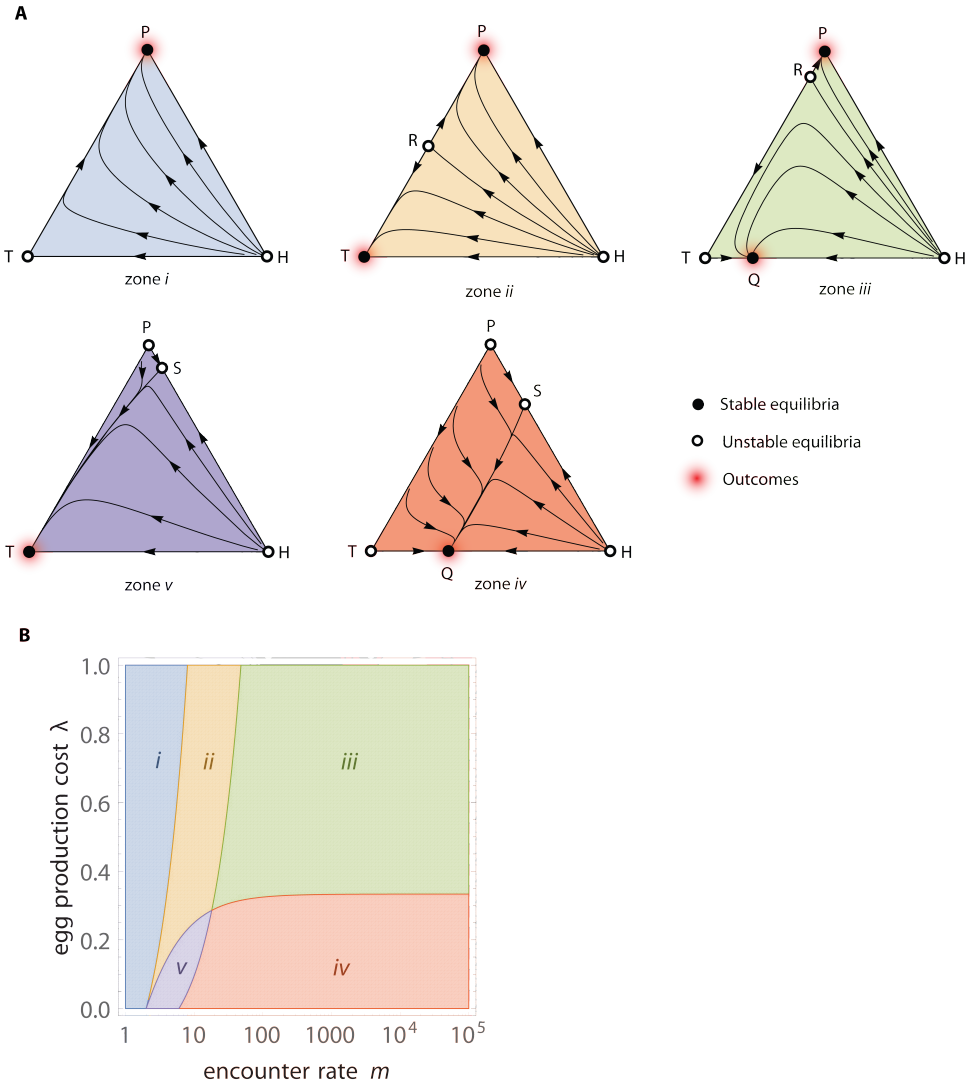

Figure 2 (**A**) Dynamical portraits characterizing evolutionary dynamics of the five regions. Each triangle represents the simplex Δ. The vertices of the simplex indicate populations that consist of only one type: T represents a population consisting of only traders, P represents a population consisting of only providers, and H represents a population consisting of only withholders. The edges of the simplex represent cases where only two strategies (the ones labeled by the two vertices connected by that edge) coexist. (**B**) Dynamic regions with unscaled estimates: egg production rate = 1, $\rho = 1$ and q = 0.5.

We can see from Figure 2b that region *v*, where eggtrading is the only evolutionarily stable outcome, occurs at sufficiently high costs of egg production (lower λ values) and low-to-intermediate encounter rates (*m*). From zone *v*, increasing encounter rates is beneficial for the withholder strategy: indeed, we enter zone *iv*, where egg traders will still invade providers, but will coexist with withholders at *Q*. This implies that at high densities of partners and intermediate levels of detection of withholders by traders, it is easier for withholders to get away with not reciprocating eggs and this strategy can thus become evolutionarily stable. From zone *v*, lowering encounter rates is beneficial for the providers strategy: indeed, we enter zone *i*, where providers invade all strategies. This implies that at low densities of partners and intermediate levels of detection of withholders by traders, providers can gain more fitness than other strategies by offering eggs without expecting reciprocation, to partners carrying eggs or not.

Effects of q and ρ

Increasing the probability *q* that traders detect and punish withholders pushes the limit separating regions *ii* and *v* from regions *iv* and *iii* towards higher encounter rates, resulting in increased sizes of zones *ii* and *v* in the parameter space (Fig. 3, comparing A1 to A2, B1 to B2, C1 to C2). This implies that when traders are better at detecting withholders, there exist more conditions allowing the stable evolution of egg trading. Increasing *q* does not change the requirements for the evolution of egg trading regarding egg production costs (no changes along the y-axis).

Assuming that eggs never become inviable (decreasing *ρ* from 1 to 0, which is

biologically unrealistic, but is shown here for the sake of understanding the model) changes all the limits separating regions and results in increased sizes of zone *v* and *ii* towards lower egg production costs, while decreasing all other zones (Fig. 3, comparing A1 to B1 and A2 to B2). On the contrary, increasing ρ from 1 to 2 (Fig.3, B1 to C1, B2 to C2) decreases the size ranges of zone *iv* and *v*, restricting them only to high costs of egg production (low λ values only), and pushing them to slightly higher encounter rates. This limits the conditions allowing the stable evolution of egg trading.

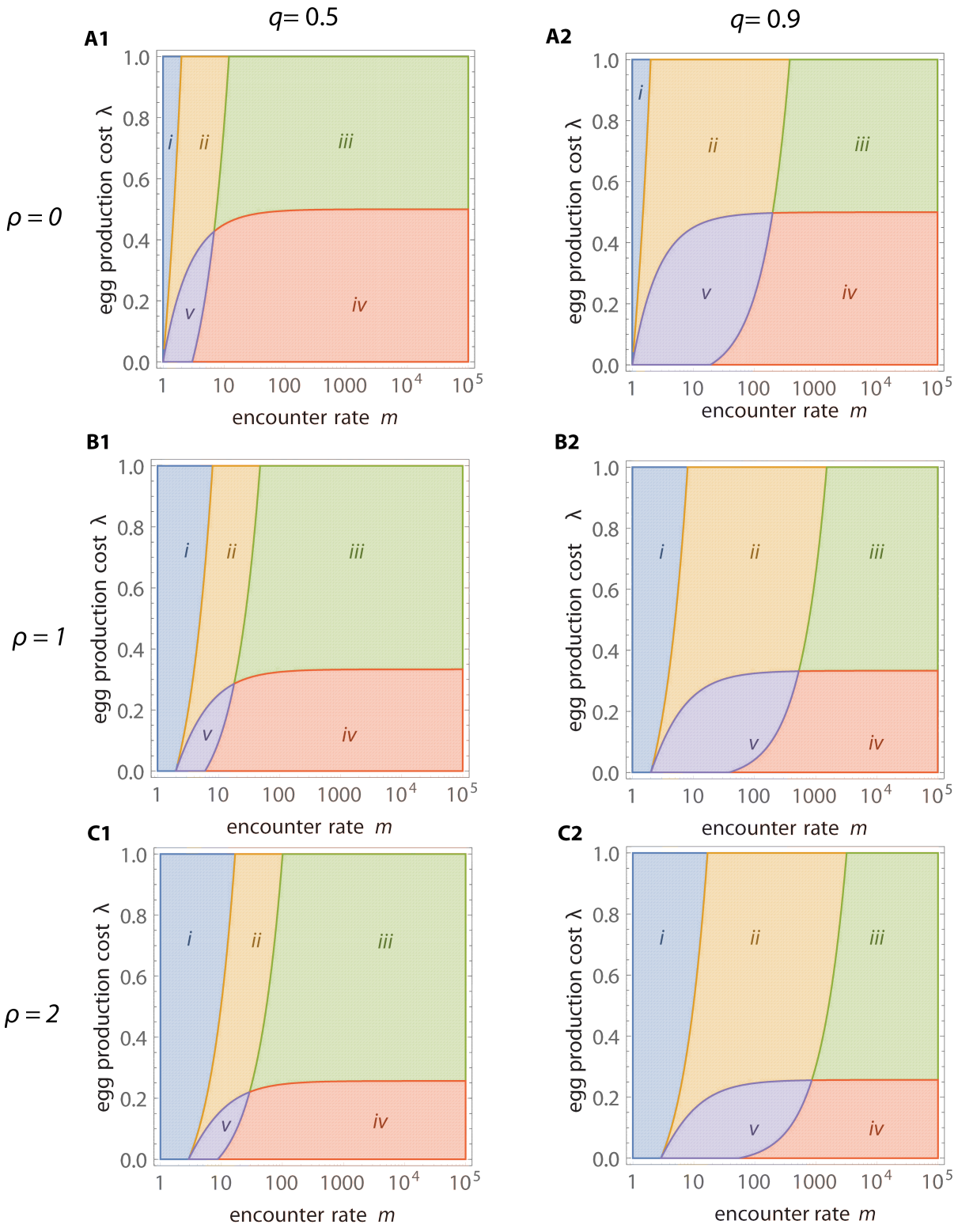

Figure 3 Dynamic regions when changing the probability of traders to detect and punish withholders (q) and the rate at which eggs become non-viable (*ρ*).

Empirical calibration

A total of 968 spawning interactions (displays, chases, pairings and matings) were observed between August 15th 2013 and June 16th 2014 among the nineteen butter hamlets present on the reef, with the vast majority of interactions (98.8%) taking place in a specific area of the reef ('spawning area', on the middle reef slope). Two fish (namely #3 and #15 in Fig. 4) were seen on the spawning area but were never seen displaying. They were thus not considered sexually active and not taken into account for the estimation of the parameters. The total number of sexually active fish in our observations (*Nsex*) was therefore 17.

The data confirm that hamlets spawn on a daily basis at the population level, with spawning activity observed on 99 of the 101 dusk dives (Fig. 4) and no apparent lunar or yearly cycle (data not shown). Figure 4 represents presence on the spawning ground through time of the 19 tagged individuals. Dots of any color represent presence on the reef, either paired (red), alone and not displaying (blue), or alone and displaying (orange). Absence of dots indicates absence from the reef. We can see that on a per-individual basis, sexually mature individuals do not show up in the mating area every day; on average, butter hamlets showed up in the spawning area on only 67.2% ± 4.3% (s.e.) of days.

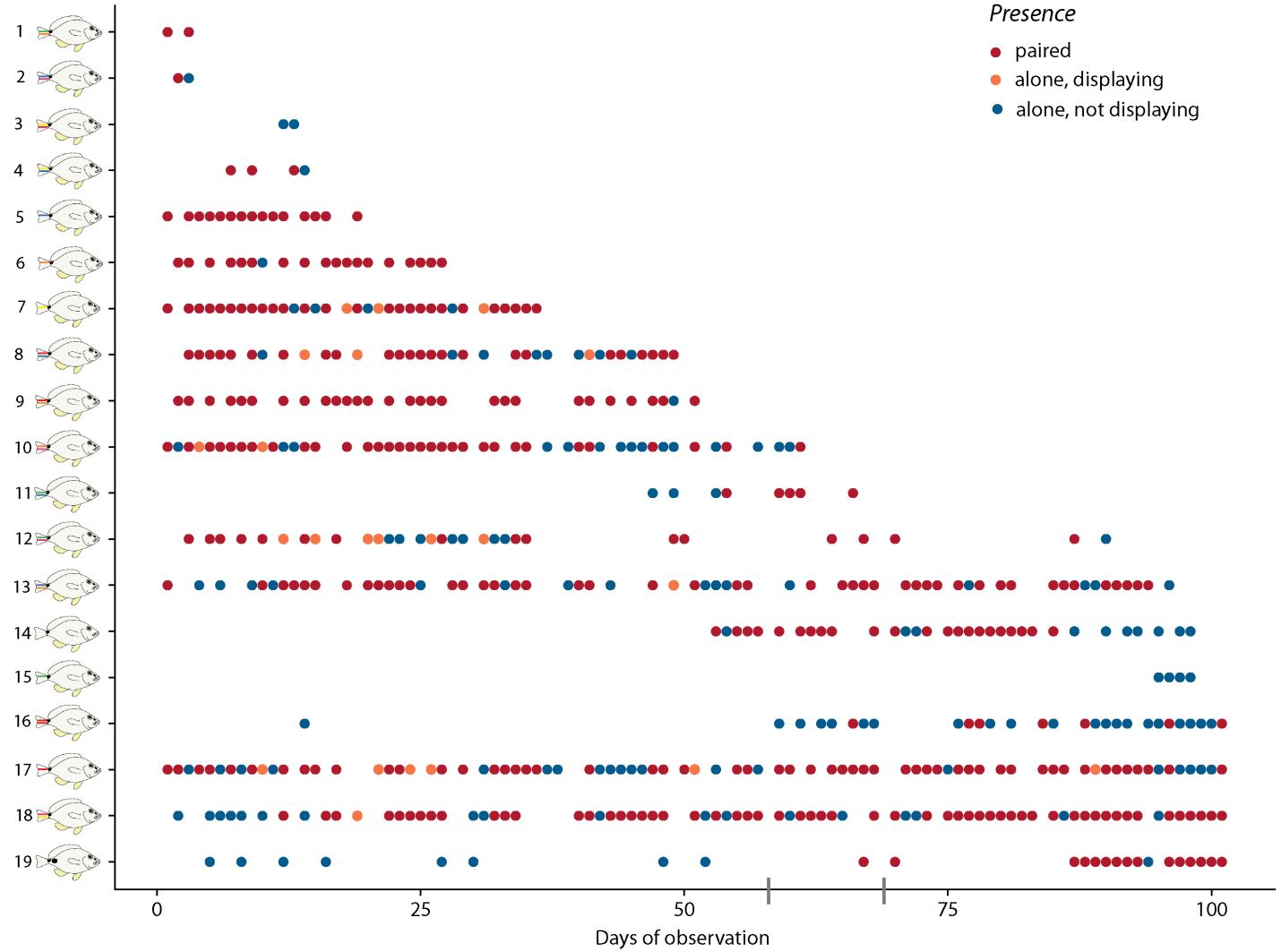

Figure 4 Presence of all tagged butter hamlets (*n*=19) at the spawning area on the reef through time, for each day of observation (*n*=101 days). Each fish is represented with the color combination it was tagged with in its caudal fin, except for two individuals who had particular natural markings which made them distinguishable (#14 in the figure did not have a black dot on the snout; #19 in the figure had a second black dot next to the large black saddle blotch on the base of the tail). Gray thin bars indicate the only two sampling days (58 and 69) where no fish were seen in the spawning area. All dots indicate presence of fish for a given day, and dot color indicates the pairing status of each fish.

The 17 fish considered to be sexually active were seen displaying and/or pairing on 52.7% ± 4.0 (s.e.) of days of their sexually active life, which we take as our egg production rate estimate (0.527 ± 0.04). Regarding the egg senescence rate, observations from Fischer (1981) indicate that ripe eggs cannot be stored overnight, meaning they are not viable after one day. Since the rate ρ at which eggs become inviable is measured relative to the rate of egg production, we took ρ = 1.896 (1 / 0.527) as our rate of egg senescence.

This implies that on average, individuals who showed up in the spawning area were not observed displaying (or pairing or spawning) on 14.5% of days.

An average of 31.9% ± 3.0 (s.e.) of the individuals who were assumed to not carry eggs (*i.e.* did not display) were present in the spawning area, implying that they had the possibility to mate in the male role in principle. We take this proportion as our λ estimate (0.319 ± 0.03), reflecting the costs of egg production in terms of mating in the male role. The encounter rate per sexually active individual per evening was 3.23 ± 0.21 (s.e.), which we take as our unscaled *m* estimate. Our final *m* estimate was 6.12 ± 0.076 (s.e.) after rescaling to an egg production rate of 1 as assumed by the model for mathematical simplicity.

The parameter *q* that captures the probability of cheaters to detect and punish withholders could not be directly translated and estimated in our dataset because observations were not pair-centered as in Fischer (1980), and do not include the complete sequence of spawnings and potential punishments by desertion that could be imposed on non-reciprocating partners. Nevertheless, we believe that hamlets can detect whether partners reciprocate at high probabilities. Indeed, when engaged in the spawning clasp,

partners assuming the male role wrap around their partner in a U-shape (Fischer 1980*a*), positioning their eyes right in front of their partner's ovipore. This strongly suggests that hamlet fish are able to visually detect whether eggs are being released, and thus know whether partners are reciprocating or not. Moreover, hamlets engage in egg parceling, whereby partners only offer small proportions of their clutch at a time for fertilization. This behavior may cut losses for fish whose partner fails to reciprocate, and may facilitate punishment by desertion as eggs are still available to be fertilized, if other mates can still be encountered. For this reason, a value of q = 0.9 was chosen as realistic to calibrate the data according to hamlet biology.

Linking the biology of hamlets to the model

Figure 5 illustrates the dynamic regions obtained with parameters estimated from empirical data on butter hamlets. The case of the egg-trading butter hamlets represented by the black dot lies in zone *i*, a zone where, according to the analytical model, conditions lead to the evolutionary stability of providers. This apparent mismatch in connecting the data with theory will be discussed in the following section.

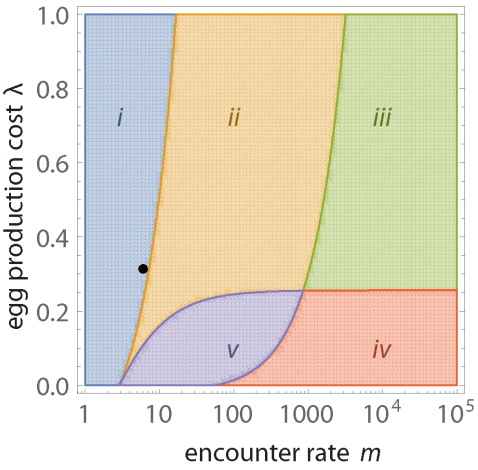

Figure 5
Dynamic regions for the model when calibrated with the empirical data: egg production rate = 0.527, ρ = 1.896, q = 0.9
Black dot represents λ and *m* estimates derived from long-term field observations of the butter hamlet, with λ = 0.319, and *m* = 6.11.

Discussion

The aim of the current study was to develop an analytical model informed by empirical data from a wild population of egg-trading reef fishes, to answer this question: how can egg trading invade a population where other mating strategies already exist?

Our analytical model shows that a combination of high opportunity costs, meaning high costs of egg production on reproductive success as a male (low λ values) allows the evolution of egg trading as the only stable outcome (zone *v* in Fig. 2b, Fig. 3 and Fig. 5). Interestingly, the model predicts that these high opportunity costs are only needed ancestrally: once the system is composed solely of egg traders, even if opportunity costs go down (λ values go up), this does not destabilize the system (this can be seen in the model as going from zone *v* to zone *ii* and remaining in T given initial frequencies).

The model also shows that some withholder detection ($q > 0$) is necessary for egg trading to evolve; it also facilitates its evolution as it increases. A higher detection of withholders allows egg trading to evolve at both relatively low and intermediate rates. The model predicts that these ranges of encounter rates are needed ancestrally and to a certain extent also for the maintenance of egg trading to prevent invasion from both withholders and providers. Indeed, at higher encounter rates, egg-traders would have to coexist with withholders (entering zone *iv*). At lower encounter rates, the model predicts egg trading to become unstable and to become invaded by providers (entering zone *i*).

The model also predicts that increasing the costs of withholding eggs (increasing *ρ)* restricts the conditions under which egg trading can evolve. Indeed, when the model incorporates the important biological fact that eggs become inviable when not fertilized, it predicts that egg trading only evolves at very high opportunity costs, and at higher encounter rates than without egg senescence. Thus, when the costs of withholding eggs

are increased, egg traders will need higher chances of finding other reciprocating partners that also carry eggs to evolve.

Previous theory considering only the two strategies of traders and providers, no egg senescence, and no egg production costs on male reproductive success, indicated that egg trading will go to fixation only if encounters with potential mates occur frequently (Henshaw et al. 2014). Our analytical model agrees that encounter rates should happen relatively frequently, although not too frequently, as that can will lead to coexistence with withholders.

Calibrating the model with empirical data collected over long-term observations from butter hamlets allowed to get a realistic sense of egg production and egg senescence rate in a species where egg trading has successfully evolved and is currently maintained. However, according to our model, the λ and m parameters estimated from the butter hamlet population lie in a region where providers should evolve as the only stable mating strategy. To resolve this dilemma between data and model, it is helpful to distinguish between the forces leading to the fixation of egg trading (what the model intends to depict), and those ensuring its maintenance (what the current data can measure in established egg traders populations). It is possible that when egg trading first evolved, ancestral populations of serranids were bigger, allowing for higher mating rates. Although simultaneous hermaphroditism itself has been predicted to be unstable at high mating rates (Charnov 1979), egg trading has also been found to stabilize simultaneous hermaphroditism once it is established in a population (Henshaw et al. 2015), and so might have evolved when populations sizes of hamlets were bigger as a stabilizing mechanism. Current work reconstructing past effective population sizes of hamlets will

help shed some light on the ancestral conditions of hamlets when egg trading must have evolved.

Taking into account the limitations of the model may also help understand the discrepancy in the fit between the model and the data. First, it is important to realize that in the model, the encounter rate intends to capture the extent of frictions in the meeting process, *i.e.* the likelihood of eggs to become inviable before a suitable partner is met. To turn this into a quantifiable parameter in natural populations, *m* was translated and measured as the number of potential mating partners each individual could have each night, which might not capture the same value intended in the model. For example, if the number of sexually active individuals is low in the population and these fish know where to meet and spawn, all eggs will be fertilized, and the encounter rate in that sense should be high in the model, although it will be low in the data. The formation of mating pairs in hamlet fish has been shown to have an important spatial component, whereby certain individuals, even from very rare speices (n = 3 on a reef), can swim hundreds of meters to well-established rendez-vous points on the reef, to meet with their preferred partners every evening and spawn, ensuring all their eggs are fertilized (Puebla et al. 2012). Incorporating this spatial and social context in the estimation of *m* could yield a value closer to what the model intends.

Another limitation of the model is that it does not take egg parceling into account: indeed, partners release and reciprocate their egg clutches but do not parcel them in the model. As mentioned in the introduction, hamlet fish engage in egg parceling, whereby they only offer a small portion of their egg clutch at a time for fertilization and take turns in doing so. Egg trading will make it easier for a fish to desert a non-reciprocating partner before that partner reaches its maximum reproductive success as a male. Indeed, fish

detecting non-reciprocators in the wild can desert them, and offer the rest of their egg clutch to other partners, therefore cutting their own losses and limiting reproductive success of fish not carrying eggs and contributing only sperm. In the model, incorporating egg parceling would imply that egg production costs on male reproductive success (λ) would be higher, as fish not carrying eggs would not be able to reach their complete male reproductive success before being detected and deserted.

Lastly, a fundamental difference between the model and the data is that the model is based on continuous time parameters, where egg production, encountering mates, and egg senescence all occur in a non-synchronized fashion. This, of course, is not the reality of most animals, and especially not of hamlets, who live in a synchronized world where different activities occur at different times, especially where mating occurs daily, in the late afternoon, before sunset. This difference generates the idea of creating a different kind of model, one which separates time scales and uses a "search-and-matching framework" to model mating interactions specific to the mating context. In this kind of setup, egg production, decisions of whether to be available for mating when not carrying eggs, and egg senescence happen in between these mating events.

Finally, why is egg trading so rare, despite its stabilizing effect on simultaneous hermaphroditism? Our model shows that conditions leading to the establishment of egg trading include high costs of egg production and intermediate encounter rates. Moreover, increased detection of non-reciprocating partners (higher q value) as well as decreased costs of withholding eggs (lower $\boldsymbol{\rho}$ values) can facilitate the evolution of this mating strategy. It is possible that the combination of these favorable conditions for egg trading may be rare in nature. The fact that trading should evolve more easily at intermediate rates raises an interesting dilemma. Indeed, the evolution of simultaneous

hermaphroditism is thought to be facilitated at low encounter rates (Charnov 1979; Puurtinen and Kaitala 2002), but it is a prerequisite for egg trading to evolve. This intertwined relationship between egg trading and simultaneous hermaphroditism may be part of the reason why egg trading is so rare. Many aspects of mating systems are subject to similar complex evolutionary feedbacks, including different conditions for the origin and maintenance of traits. As pointed out in Henshaw et al. (2015), more thought needs to be put into disentangling these density-dependent induced changes in selection in these systems (Lehtonen and Kokko 2012).

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SYNTHESIS AND PERSPECTIVES

The current thesis aimed at investigating processes underlying the spread and diversification of novelty in hamlet fish, using the hamlets as a model system. To do so, I used a combination of genomic, behavioral and theoretical approaches to address novelty from three different angles and at three different levels of biological organization. In Chapter I, I looked at the signature of novelty at the level of the genome: what is the underlying genomic basis of two processes driving novelty, namely local adaptation and speciation, across species and populations of hamlet fish, and are these architectures comparable? In Chapter II, I focused on intraspecific phenotypic novelty in behavior at the individual level: do butter hamlets consistently differ in aggressive mimicry behavior, and if so, are these consistent individual differences linked to mate choice? In Chapter III, I zoomed out of the hamlet radiation to investigate the evolutionary dynamics leading to the invasion of egg trading, a rare mating system shared by some simultaneous hermaphrodites, including hamlets: what are the evolutionary game dynamics that support the successful invasion and persistence of egg-trading in a population composed of different mating strategies? In this section, I will sum up the main advances of each chapter and give an outlook on potential future research directions that could help strengthen our understanding of the evolution of these novelties in hamlet fish.

Traditionally, the study of local adaptation and the study of speciation have been prominent research avenues focusing on the evolution and diversification of novel traits (Wagner and Lynch 2010), and on connecting genes to phenotypes. Over the last decade, advances in next-generation sequencing for non-model organisms have started to yield important insights showing that the evolutionary success of both these processes depends in part on a population's genetic and genomic properties: for instance, the genomic

architecture underlying the expression of adaptive traits can constrain or facilitate their response to selection (Nosil 2012; Chaves et al. 2016); reciprocally, the action of selection can shape this architecture (Savolainen et al. 2013; Arnegard et al. 2014; Seehausen et al. 2014; Soria-Carrasco et al. 2014). Therefore, knowing the genomic architectures of these processes can greatly enhance our overall understanding of the evolution and diversification of novel traits.

In Chapter I, we showed that, at the resolution attainable through RAD-sequencing, the genomic architectures of local adaptation and of the early phases of speciation do not differ fundamentally: they are both characterized by one or a few genomic islands of differentiation (repeated outliers) against a background 'sea level' of almost no differentiation. Indeed, the 97,962 SNPs genotyped in the 126 sampled individuals provided very similar genome-wide levels of divergence within species (F_{ST} estimate = 0.0042) and between species (F_{ST} estimate = 0.0038) as well as very similar population genetic clustering and phylogenetic analyses patterns. These results parallel the population genetic patterns reported in other recently diverged taxa such as East African cichlids (Seehausen et al. 2008; Wagner et al. 2012), Darwin's finches (De León et al. 2010; Chaves et al. 2016), stick insects (Nosil et al. 2012), and the rough periwinkle (Ravinet et al. 2016), where divergence among populations within species or ecotypes can be comparable to divergence among species or ecotypes, although no studies, to our knowledge, have explicitly contrasted the population genomic patterns along these two axes of divergence.

Even though the number of repeated outliers involved in local adaptation ($n = 3$) was comparable to the one involved in speciation ($n = 1$), different sets of loci appeared to be involved in the different processes. Indeed, a tropomyosin locus (*Tmp4*) (along with

two anonymous loci) emerged as a candidate involved in local adaptation whereas a *Hox* locus emerged as a candidate involved in speciation. *Hox* genes, apart from playing a major role for the anterior–posterior patterning of tissues along the body axis during development, have been shown to be redeployed later in development to play a role in terminal color pattern phenotype in *Drosophila* and Satyrinae butterflies (Jeong et al. 2006; Saenko et al. 2011), strongly suggesting they could be involved in color pattern in the hamlets too (Puebla et al. 2014). Tropomyosin, on the other hand, is a protein playing an important role in muscle contraction and has been associated with plasticity in the pharyngeal jaw apparatus of cichlids (Gunter et al. 2013). *Tmp4* has also been found to be upregulated in the common carp under cold water temperatures (Gracey et al. 2004). It is thus tempting to hypothesize that the outlier signals at the *Tmp4* locus could be linked to local adaptation to colder water temperatures, which occur in Belize. Future studies focusing on fine mapping of the association of *Hox* and species differences in color, as well as of *Tmp4* and population differences, are needed to refine these hypotheses.

Since RAD-sequencing can only give a partial representation of the genome, a natural next step when investigating the genomic basis of local adaptation or speciation in the hamlets will be a whole-genome re-sequencing study, filling the gaps left open by RAD. Since the Picq et al. (2016) study was published, high density egg- and sperm-specific linkage maps (Theodosiou et al. 2016) have been generated. In addition, a high quality chromosome level assembly of the barred hamlet genome (Hench 2017) has been put together, which can now serve as a reference for future whole-genome based studies on the serranids. The assembly has a total size of 612 Mb, and resolves all 24 linkage groups (and the mitochondrial genome), that were expected from the linkage maps on hamlets (Theodosiou et al. 2016). As a first step towards gaining a better resolution of the

level of genomic divergence within and between hamlet species, the RAD dataset used in the Puebla et al. (2014) and the Picq et al. (2016) studies was re-analyzed using the newly assembled genome as a reference (figure 2, from Hench 2017)).

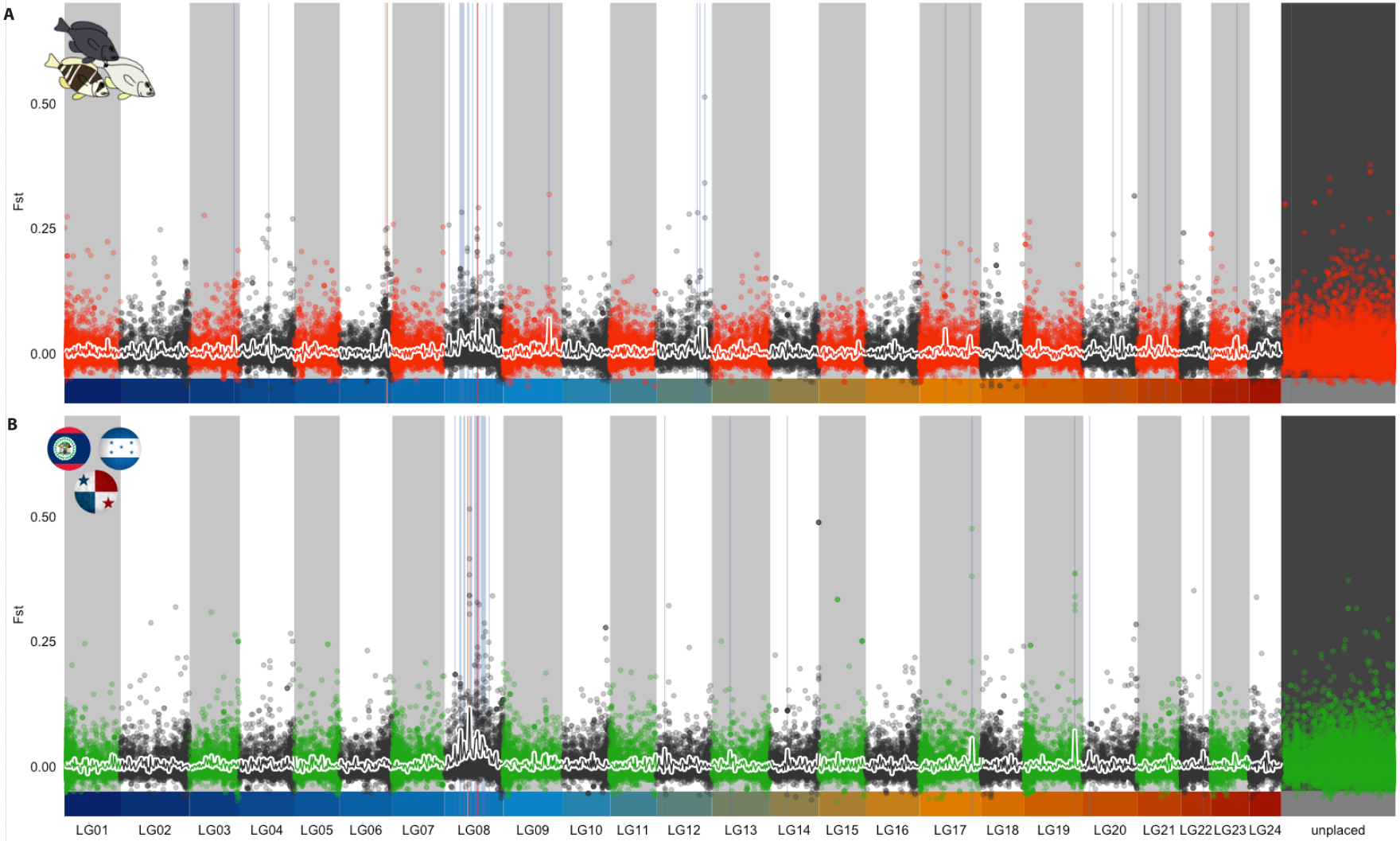

Figure 2 Comparison of global F_{ST} values for speciation and adaptation scenarios. Dots represent original F_{ST} values for SNPs genotyped through RAD sequencing and the white line shows a smoothed F_{ST}, averaged over non-overlapping 450kb windows. The colored bars mark outlier windows (F_{ST} > 99% quartile), blue bars indicate windows that are not shared between comparisons, orange bars indicate windows that are shared and the red bar shows windows that are shared as well as repeated (**A**): F_{ST} values for the speciation scenario. (**B**): F_{ST} values for the adaptation scenario

The re-analysis with the reference genome allowed positioning of the *Tpm4* locus on linkage group 8 and the *Hox* locus on linkage group 12. Interestingly, the *Tpm4* locus was found within a 20Mb region on linkage group 8 that shows very little recombination in the linkage maps provided by Theodosiou et al. (2016). Areas of low recombination are possibly inversions, which are hypothesized to present a mechanism to reduce gene flow and enhance divergence between inverted and non-inverted haplotypes (Sodeland et al. 2016). It has been shown that, for other teleost species like the cod (Sodeland et al. 2016) or the stickleback (Marques et al. 2016), divergence accumulates especially in areas associated with large inversions. Therefore the detection of a large area of low recombination (possibly an inversion) on linkage group 8 might be of special relevance when investigating the genomic architecture of local adaptation within the hamlets.

Nevertheless, while providing genome-wide information, RAD data still remains 'patchy' and does not allow to specifically pinpoint a specific gene model to the definite cause of a peak, as peaks in F_{ST} form RAD sequencing represent their closest restriction site and not the exact loci under selection. A current whole-genome re-sequencing study, including 12 individuals of barred, black, and butter hamlets, repeated geographically in the same locations as the Picq et al. (2016) study is currently underway, and will be pivotal in unraveling the more detailed genomic architectures of local adaptation and early stages of speciation in hamlets.

While Chapter I focused principally on the genomics of novelty between and within species, Chapter II focused essentially on phenotypic novelty, through detailed behavioral, ecological and pairing observations at the individual level in butter hamlets. Butter hamlets were found to differ consistently in how much they engage in aggressive mimicry behavior, forming

two discrete behavioral types, or alternative behavioral phenotypes, that also differ consistently with respect to foraging, territoriality, and mate choice behavior. 'Aggressive mimics' were found to eat significantly more glass gobies and leave their territories more often than 'territorials', who rarely associate with the foureye butterflyfish models, feed significantly more on mysids, and restrict most of their feeding to their home territories. Moreover, pairing observations of these individuals revealed that mating tends to be assortative with respect to behavioral type, suggesting that aggressive mimicry behavior plays an important role in mate choice.

In order to better understand how these behavioral types may coexist and persist through time, future studies could focus on several points: first, the genetic basis and heritability of variation in aggressive mimicry behavior could be investigated, in order to understand whether these behavioral types are direct targets of selection. In great tits, behavioral variation in exploratory behavior has been shown to be heritable (Dingemanse et al. 2002) and to have a genetic basis (Fidler et al. 2007). Nonetheless, even without a genetic basis, aggressive mimicry behavior could persist through time by being a learned trait: indeed, learning has been found to play an important role in the development of individual behavioral differences in birds (reviewed in Giraldeau (1984)), insects, mammals, and fishes (Clark and Ehlinger 1987; Magurran 1993). As pointed out by West-Eberhard (2003, chapter 18), learning can be a potent source of adaptive evolution by recurrently generating advantageous morphology-behavior combinations (tracking behavior in our case) that can become subject to selection, and then to genetic accommodation. Future experiments could try to determine how successful hamlets are at learning tasks, such as associating particular behaviors with rewards.

Directly quantifying the benefits accrued by each behavioral strategy would greatly enhance our understanding of how these alternative strategies can coexist, and whether or not they guarantee similar payoffs. Indeed, potential fitness benefits accruing to 'aggressive mimics' specifically include better food sources attained through engaging in aggressive mimicry behavior. In turn 'territorials', by staying within their territories, may gain benefits of increased protection from predation and aggression from other hamlets present on the reef. Understanding whether the fitness payoffs of each behavioral types are at equilibrium would help us understand the conditions allowing these behavioral types to coexist. Fitness tradeoffs between alternative phenotypes have been demonstrated in a large variety of organisms, such as in horned and hornless beetles, where large horned males have a clear advantage in fights, whereas small hornless males are faster at running through tunnels where they sneak copulations (Emlen 1997; Moczek and Emlen 2000).

Moreover, it would be crucial to understand whether densities of the model species influence the coexistence of the two behavioral types. Indeed, since aggressive mimicry itself is maintained though frequency-dependent selection, the aggressive mimic behavioral type cannot be obligate, as this could lead to a collapse of the mimic-model system, whereby preys could learn to discern the predatory butter hamlets (mimics) from the non-predatory foureye butterflyfishes (models). Repeating detailed behavioral observations of butter hamlets across reefs with varying densities of the foureye butterflyfish would inform us on whether the occurrence of different behavioral types depends on the density of models.

Most interestingly, such detailed behavioral, ecological, and pairing observations studies should be extended to other species of the *Hypoplectrus* radiation, which have been hypothesized

to also be aggressive mimics. A great candidate would be the blue hamlet *H. gemma,* a putative mimic of the blue chromis *Chromis cyanea*, for which some behavioral data in relation to the mimicry hypothesis is available (Randall and Randall 1960).Such comparative studies would allow us to determine how recurrent this variation in behavioral type is among hamlet species, and what role it could have played across the entire *Hypoplectrus* radiation.

A pattern in which intraspecific variation parallels species differences has been found to be a key characteristic of other well-studied adaptive radiations such as East African cichlids (Kornfield et al. 1982) or Darwin's finches (Werner and Sherry 1987) and has been proposed to be indicative of diversification through developmental plasticity, whereby new forms emerge from plastic development in the ancestral phenotype, referred to as the 'flexible stem' (West-Eberhard 2003, 2005). According to this flexible stem model of evolution, the ancestral lineage might have been particularly phenotypically plastic in the development of key traits, that is adaptive traits that are especially diverse in derived lineages and probably contributed to diversification. The idea that phenotypic plasticity may precede and facilitate evolutionary adaptation has recently gained attention (Ghalambor et al. 2007; Levis and Pfennig 2016; Schneider and Meyer 2017) and has been directly tested and supported in sticklebacks (Wund et al. 2008). It is possible that plasticity in the development of aggressive mimicry behavior of the flexible stem of hamlets could have facilitated the radiation into species that associate with models and species that do not. Tests of this mode of evolution would require knowing what the ancestral stem group was like in hamlets, a question that is still not fully resolved. However, exploring how recurrent this pattern of intraspecific variation paralleling inter-species differences

in different hamlets species would be a first step towards elucidating whether the hamlet radiation may have been driven by this mode of evolution.

Chapter III shifts focus to investigate the evolution of their rare mating system of egg trading. This study integrates evolutionary game theory and empirical data to investigate the conditions leading to the invasion of egg trading in a market composed of two other mating strategies. Our model showed that egg trading becomes evolutionarily stable at high opportunity costs of egg production (indicating that individuals cannot be producing eggs and gaining reproductive success in the male role at the same time) and at intermediate encounter rates. When incorporating costs of withholding eggs (*i.e.* incorporating egg senescence, a biologically realistic assumption), our model indicates that even higher opportunity costs and slightly higher encounter rates will be necessary for egg trading to evolve. This implies that when eggs are costly to produce (as they become inviable), egg traders may need to ensure that they encounter enough partners who will have to reciprocate eggs in order to gain enough reproductive success. Calibrating the model with empirical data collected over long-term observations from butter hamlets allowed to get a realistic sense of egg production rate and egg senescence rate in a species where egg-trading has successfully evolved and is currently maintained. However, the apparent mismatch between the model and data that emerged when estimating the encounter rate and egg production costs of the butter hamlets highlighted the need to clearly distinguish the forces leading to the initial establishment of egg trading and the forces underlying its maintenance. With regards to hamlets specifically, a model that incorporates the sequence at which egg production, mating, and egg senescence occur through a "search and match

framework" might prove more accurate and thus more powerful in predicting how this mating system evolved.

To summarize, this thesis adopted a multifaceted approach to investigate the spread and diversification of novelty in hamlet fish, in order to understand how changes occur at the level of the genome, the phenotype, and of populations. Altogether, the results of this work aim to portray that evolutionary change leading to novelty is of an intertwined nature. Understanding factors initiating change in the genome (that may, or may not, cause phenotypic change) and the level of genomic diversity that exists within and between species is necessary to understand the potential of populations to evolve. From another perspective, detailed behavioral and ecological studies at the phenotypic and individual level can add additional directions to the evolution of individuals and to the causation of phenotypic variation. Lastly, the comprehensive framework of evolutionary game dynamics on evolution clearly embodies the fundamental principle that the fitness of an individual is not constant but always depends on the relative proportions of others in the population.

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References for illustrations:

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SUPPORTING INFORMATION

SUPPORTING INFORMATION FOR CHAPTER I

Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp, Serranidae)

Sophie Picq, W. Owen McMillan, Oscar Puebla

Supporting Table S1. Summary of the 5 assemblies with different combinations of *m* (stack depth) and *M* (mismatch) parameters. As expected (Catchen *et al*. 2013), the number of stacks decreases with increasing *m* and *M* parameter values. Assuming a 1Gb genome typical of many serranids and a GC content of 41% (from the paired-end reads), one would naively expect 6,459 *Sbf*I cut sites in *Hypoplectrus* $(0.41/2)^6$ x $(0.59/2)^2$ x10^9. This is four times less than the 26,906 sites suggested by our main assembly (*m*=3 *M*=2). Such discrepancies are not rare (e.g. 3,221 expected *Sbf*I cut sites versus 22,830 observed in the stickleback genome, Hohenlohe *et al*. 2010), and stress the importance of performing a pilot study when planning RAD studies on an organism for the first time or with a new restriction enzyme. References in the main text.

	***m*=3 *M*=2**	***m*=3 *M*=3**	***m*=4 *M*=2**	***m*=5 *M*=4**	***m*=10 *M*=4**
Mean n. of stacks per sample	53,811	53,420	50,133	46,506	36,103
Global F_{st} among populations	0.0042	0.0043	0.0042	0.0044	0.0044
Number of outliers	107	104	113	106	76
Proportion of outliers (%)	0.07	0.06	0.07	0.07	0.06

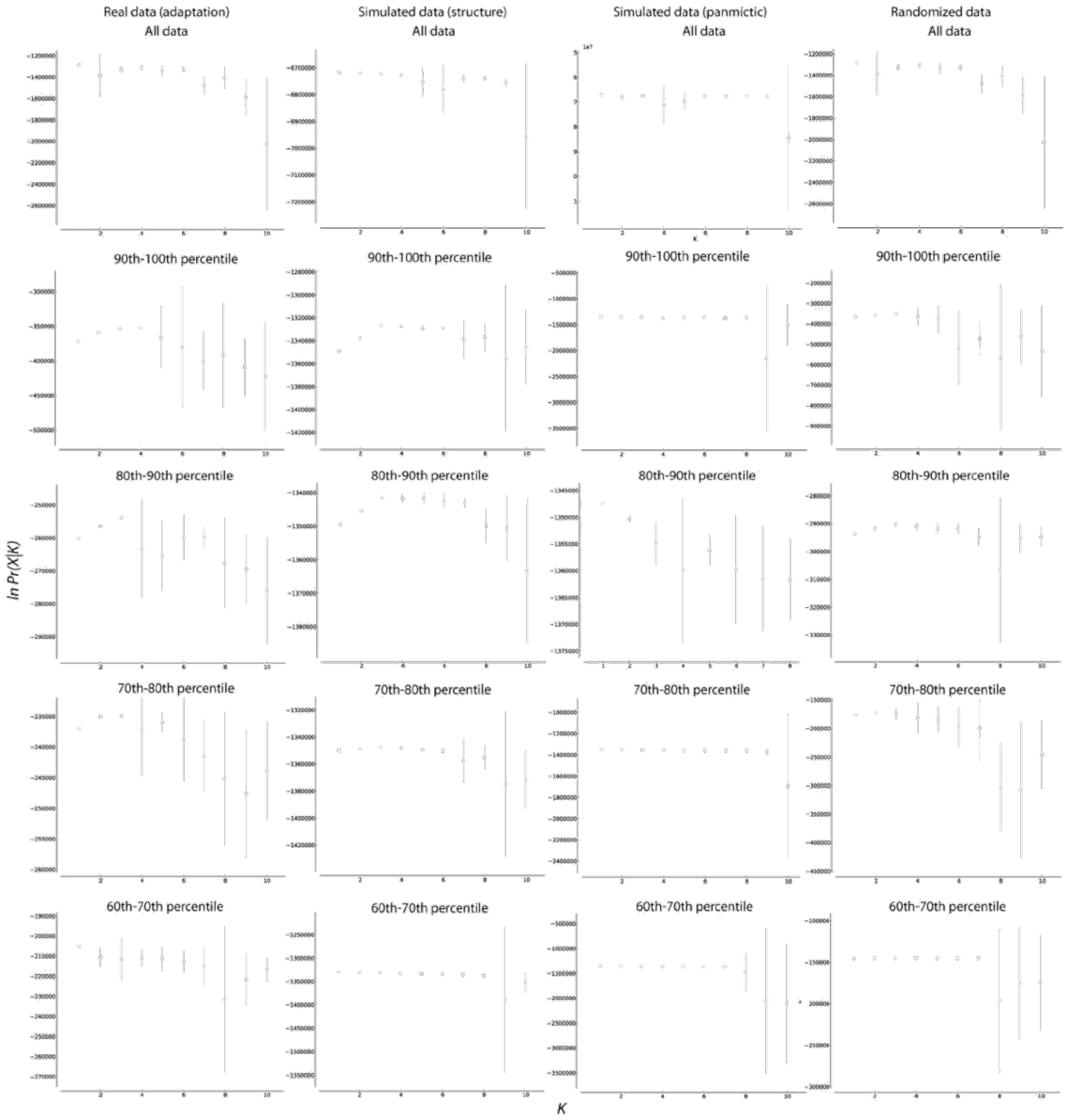
Supporting Table S2. Mean number of individuals sampled per site, observed and expected heterozygosity, nucleotide diversity (π) and F_{is} in the three locations considered in this study.

Species	Population	Mean *n*	Obs. het.	Exp. Het.	π	*Fis*
All sites						
H. nigricans	Belize	11	0.00184	0.00233	0.00247	0.00173
H. puella	Belize	11	0.00183	0.00240	0.00253	0.00195
H. unicolor	Belize	9	0.00165	0.00221	0.00236	0.00192
H. nigricans	Honduras	12	0.00185	0.00244	0.00257	0.00202
H. puella	Honduras	12	0.00192	0.00248	0.00260	0.00196
H. unicolor	Honduras	6	0.00154	0.00192	0.00210	0.00137
H. nigricans	Panama	6	0.00144	0.00196	0.00217	0.00174
H. puella	Panama	6	0.00134	0.00188	0.00207	0.00176
H. unicolor	Panama	11	0.00169	0.00229	0.00242	0.00204
All	Belize	31	0.00179	0.00241	0.00245	0.00262
All	Honduras	30	0.00189	0.00243	0.00248	0.00234
All	Panama	20	0.00155	0.00208	0.00214	0.00214
H. nigricans	All	26	0.00184	0.00236	0.00241	0.00219
H. puella	All	29	0.00186	0.00238	0.00242	0.00222
H. unicolor	All	26	0.00167	0.00228	0.00233	0.00255
All	All	79	0.00178	0.00239	0.00240	0.00335
Variant sites						
H. nigricans	Belize	11	0.099	0.125	0.132	0.093
H. puella	Belize	11	0.098	0.129	0.136	0.105
H. unicolor	Belize	9	0.087	0.117	0.125	0.101
H. nigricans	Honduras	12	0.100	0.131	0.138	0.109
H. puella	Honduras	12	0.104	0.134	0.141	0.106
H. unicolor	Honduras	6	0.083	0.104	0.114	0.074
H. nigricans	Panama	6	0.070	0.095	0.105	0.085
H. puella	Panama	6	0.071	0.099	0.109	0.093
H. unicolor	Panama	11	0.090	0.122	0.129	0.108
All	Belize	31	0.095	0.128	0.130	0.139
All	Honduras	30	0.100	0.129	0.131	0.124
All	Panama	20	0.082	0.110	0.113	0.114
H. nigricans	All	25	0.096	0.124	0.126	0.115
H. puella	All	28	0.097	0.125	0.127	0.117
H. unicolor	All	25	0.087	0.118	0.121	0.132
All	All	79	0.093	0.124	0.125	0.174

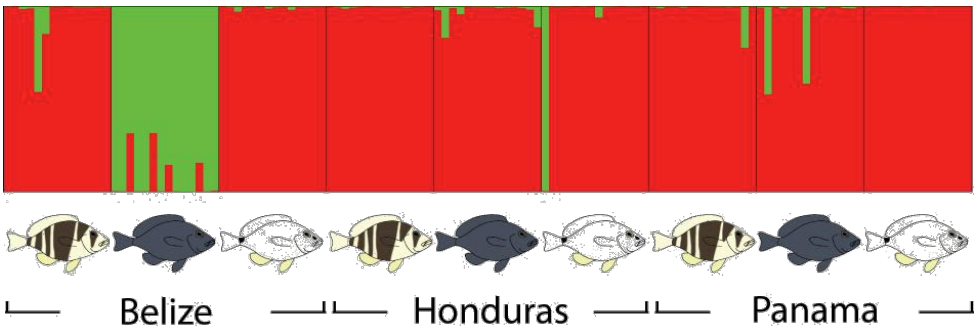
Supporting Table S3. Highest blast hits for the stacks containing non-repeated outlier SNPs. Only hits with a E-value <1E-06 are shown.

stack ID	gene	E-value
17390	*brd1a*	8E-08 (cave fish)
18769	*EPHB3*	3E-06 (platyfish)
21286	*mpnd*	1E-19 (stickleback)
23988	*adcy1a*	2E-36 (tetraodon)
26199	*COMP*	4E-15 (stickleback)
28418	*tpm4*	2E-31 (stickleback)
31152	*ccbl2*	9E-35 (tilapia)
33666	*cntn3a*	9E-08 (zebrafish)
39824	*SZT2*	8E-29 (tilapia)
43013	*jak2*	4E-33 (Amazon molly)
46473	*lysosomal protective protein-like*	6E-28 (croceine croaker)
53341	*MYO15B*	6E-19 (stickleback)
58757	*slc35c2*	8E-12 (stickleback)
59458	*mthfd1b*	1E-27 (platyfish)

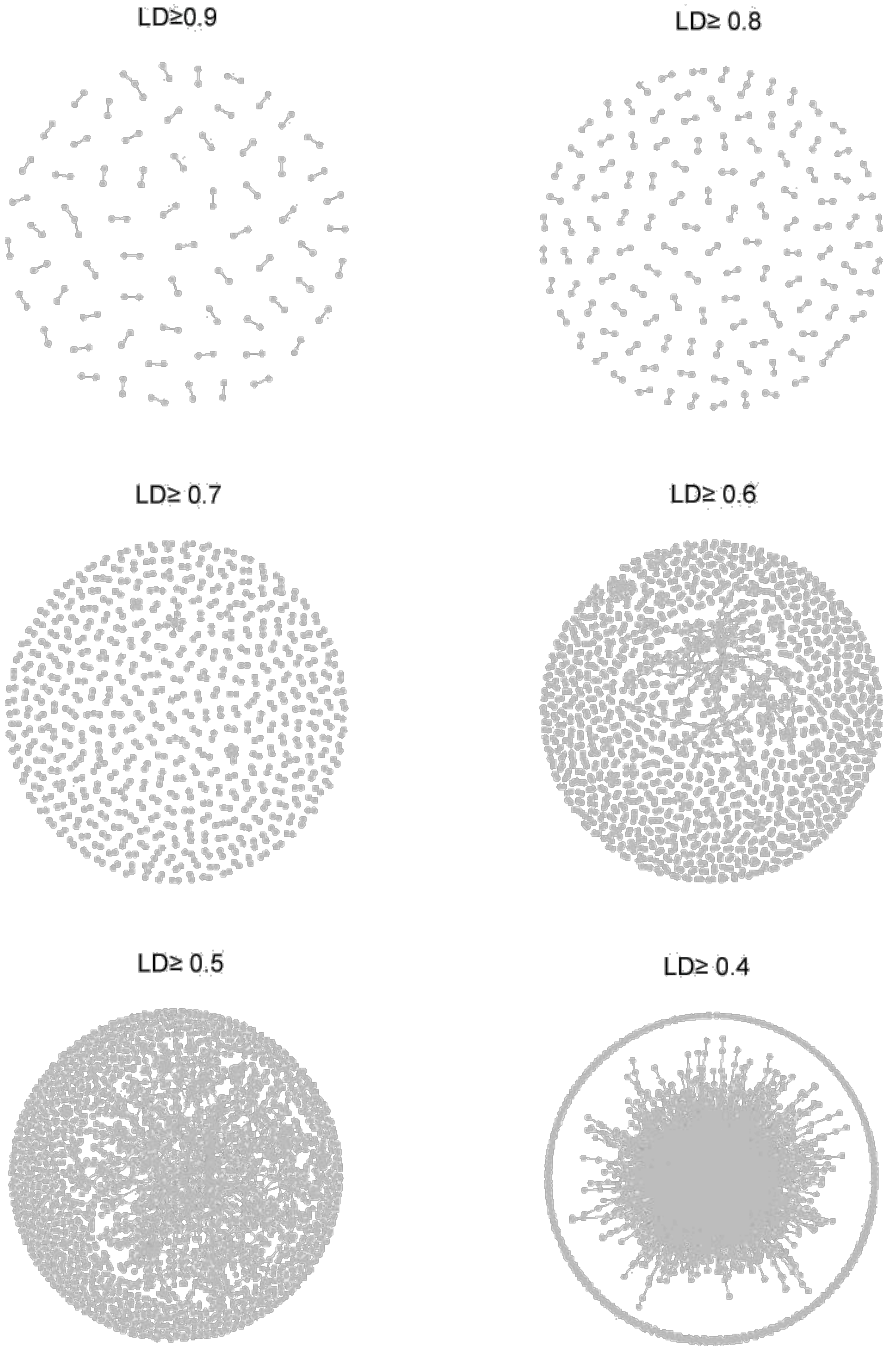
Supporting Figure S1. Clustering results for adaptation (between Belize, Honduras and Panama), simulated data (panmictic, migration rate m=0.5 and structure, migration rate m=0.02), and randomized data. In each case the entire dataset (~40,000 SNPs) is presented above, followed by the SNPs above the 90th F_{st} percentile, between the 80th and 90 th F_{st} percentiles, between the 70 th and 80th F_{st} percentiles, and between the 60th and 70th F_{st} percentiles (~8,000 SNPs in each case). No clustering was found when considering the SNPs below the 60th percentile (data not shown).



Supporting Figure S2. Clustering pattern obtained with *K*=2 when considering all the data and removing rare alleles (present in only one individual per location). The same pattern was observed in the 10 replicate runs, although each run was started with a different seed number.



Supporting Figure S3. Patterns of linkage disequilibrium among the 10,734 SNPs considered in the LD analysis. Vertices represent loci and edges LD values that are above a given threshold (indicated above each plot). A small proportion of SNPs (249 out of 10,734) present LD values ≥ 08, the large majority of which involve a single pair of loci. These may be on flanking regions of the same restriction sites, as a single SNP per stack was used for these analyses. Larger clusters emerge, grow and merge at lower LD values.



SUPPORTING INFORMATION FOR CHAPTER II

Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population

Appendix part A: Differences in foraging behavior between the two behavioral types

In order to test for differences in foraging behavior between i. the two behavioral clusters, ii. the two behavioral clusters while tracking the foureye butterflyfish specifically and iii. the two behavioral clusters while alone specifically, we applied the same Poisson-lognormal multivariate GLMM analysis described in the Methods using the MCMC.OTU package (Matz 2016). Model priors, number of iterations, thinning length and burn-in were as described in the Methods.

For the differences in foraging behavior between the two behavioral clusters, foraging bout counts per food category throughout the totality of each observation period (45 min) was considered as the multivariate response variable. Each count was expressed relative to the total count of bouts for each observation period. Behavioral type identity ('aggressive mimic' or 'territorial') was considered as a fixed effect and fish identity as a random effect. Pairwise comparisons between behavioral types were made for each food category and tested for statistical significance as described in the Methods.

For the differences in foraging behavior between the two behavioral clusters while tracking specifically, foraging bout counts per food category while tracking for each observation period was treated as the multivariate response variable. Each count was expressed relative to the total counts of bouts performed while tracking for each observation period, which accounts for the fact that individuals vary with respect to the amount of time spent tracking. Behavioral type ('aggressive mimic' or 'territorial') was considered as a fixed effect and fish identity as a random effect. Pairwise comparisons between behavioral types while tracking were made for each food category and tested for statistical significance as described in the Methods.

A similar approach was taken for the differences in foraging behavior between the two behavioral clusters while alone specifically. Foraging bout counts per food category while alone for each observation period was treated as the multivariate response variable. Each count was expressed relative to the total counts of bouts performed while alone for each observation period, which accounts for the fact that individuals vary with respect to the amount of time spent tracking. Behavioral type ('aggressive mimic' or 'territorial') was considered as a fixed effect and fish identity as a random effect. Pairwise comparisons between behavioral types while alone were made for each food category and tested for statistical significance as described in the Methods.

Appendix part B: Individual-based model

B1. The ODD protocol

We provide the description of the reference model used in the manuscript by following the ODD (Overview, Design concepts, Details) protocol format (Grimm et al. 2006, 2010; Scotti et al. 2017). Unused concepts are omitted in the description.

Overview

(1) Purpose of the model

The model simulates pairing among 'aggressive mimic' and 'territorial' butter hamlets under a null model of random pairing, taking into account the presence/absence of each fish in the spawning area as observed in the field as well as the different behaviors of the two groups. As described in the Results, the 'territorials' tend to meet at specific 'rendezvous' sites in the spawning area while the 'aggressive mimics' tend to swim over the entire spawning area. The objective is to test whether the level of assortative pairing among the 'aggressive mimics' under this null model differs from what was observed in the field.

(2) Entities, state variables and scales

- Agents: 3 'aggressive mimic' and 8 'territorial' butter hamlets are the agents included in the model. State variables that describe each agent are: behavioral type ('aggressive mimic' or 'territorial'), individual identity (codes are coherent with the individual tags in the field), grid cell (to define location in the spawning area), velocity (zero for the 'territorials' and 30 cm s^{-1} for the 'aggressive mimics'), pairing status (i.e. whether individuals are involved in pairing, in which case they do not move), identity of the pair (to distinguish between assortative and disassortative pairing) and time of pairing (to know when the 'aggressive mimics' start pairing).
- Spatial units: a square grid of 71 m x 71 m represents the spawning area, with a cell resolution of 1 m x 1 m. The grid is wrapped to form a torus by connecting opposite edges to avoid edge effects. State variables that describe each cell are: type (to identify the 'rendezvous' sites), list of agents that are present (based on behavioral type and individual identity), occurrence of pairing (yes/no), and number of pairing events (counts). 'Rendezvous' sites are squares of 5 m x 5 m.
- Time units: one time step represents 3.3 seconds and each simulation lasts for one hour (1080 discrete time steps). We adopted a time scale of one hour to be consistent with the average time of real evening dives, which cover the pairing period.

(3) Process overview and scheduling

All agents can ***pair*** if they occupy the cell of another free agent (i.e. an agent that is not already paired). 'Territorial' butter hamlets are static and occupy 'rendezvous' sites only. 'Aggressive mimics' ***move*** following a random pattern, but stop moving when pairing occurs. Time is modeled as discrete steps during which discrete events can occur. State variables are updated in a synchronous way (i.e. the new values are stored until all agents have executed the process, and then all are updated at once).

Design concepts

(1) Basic principles

The model simulates pairing among agents that belong to two groups: 'aggressive mimic' and 'territorial' butter hamlets. It has been observed in the field that the 'aggressive mimics' and the 'territorials' have distinct behaviors. 'Aggressive mimics' move in the search of partners, while 'territorials' have preferential 'rendezvous' sites and display a more static behavior. The simulations are used to quantify the level of assortative pairing among the 'aggressive mimics' specifically. In particular, we aimed to characterize the level of assortative pairing that is expected among the 'aggressive mimics' if they move randomly in the spawning area. This level is then compared with the real data to test whether the level of assortative pairing observed in the field differs from random expectations.

(2) Emergence

The simulations are used to compute the number of assortative and disassortative pairings between the two behavioral clusters. Such numbers are computed after each set of 42 simulation runs (reflecting the 42 evening dives). The model is executed for 1000 simulation sets (of 42 runs each) and allows defining the frequency distributions for the number of assortative and disassortative pairings. In particular, the frequency distribution of assortative pairing among the 'aggressive mimics' is compared with the number of assortative pairings observed for the same group in the field. The chances of pairing depend on the presence of free agents, which in turn is related to the probability of each agent to be included in the simulation run. Thus, presence/absence, availability of free agents and pairing are influenced by the frequency of appearance of hamlets in the spawning area as observed in the field.

(3) Adaptation

The 'aggressive mimics' move randomly until they enter a cell occupied by a free agent (i.e. either another 'aggressive mimic' alone or a 'rendezvous' site with an unpaired 'territorial'). 'Aggressive mimics' stop moving until the end of the simulation run when pairing occurs.

(4) Objectives

The objective of each agent is to find a partner to pair with. Two alternative strategies are adopted to achieve such objective: (A) static presence in the spawning area, with occupation of 'rendezvous' sites ('territorials') or (B) dynamic behavior, with random moves in the whole spawning area ('aggressive mimics').

(5) Sensing

The 'aggressive mimics' are able to detect each other when they occupy the same cell (size = 1 m x 1 m). All agents sense the 'territorials' when they enter the 'rendezvous' sites (size = 5 m x 5 m). Both the 'aggressive mimics' and the 'territorials' are bounded to the spawning area of size 71 m x 71 m (5041 m^2).

(6) Interaction

Two 'aggressive mimics' pair when they occupy the same cell. When the 'aggressive mimics' enter a 'rendezvous' site and find an unpaired 'territorial' they pair with it.

'Territorials' pair with each other if they are on the same 'rendezvous' site. Pairing always occurs when free agents meet.

(7) Stochasticity
Random numbers are used to initialize the spatial coordinates of four 'rendezvous' sites and place the 'aggressive mimics' and 'territorials'. The 'rendezvous' site where each 'territorial' agent is located is randomly selected. Random numbers are used to initialize the spatial coordinates of the 'aggressive mimics' and define their movement direction.

(8) Observation
Each simulation set is composed of 42 runs, which correspond to the number of evening dives executed in the field. At the end of each simulation set, the numbers of assortative and disassortative pairings are recorded. Altogether, we performed 1000 complete simulation sets (of 42 runs each), which allow generating the frequency distribution of the numbers of both assortative and disassortative pairings. In particular, the frequency distribution of assortative pairings among 'aggressive mimics' is compared with its empirical counterpart (i.e. the number of pairings observed over the 42 dives). This test aims at identifying whether the level of assortative pairing among the 'aggressive mimics' observed in the field follows a random pattern or deviates significantly from it.

Details
(1) Initialization
At the beginning of each simulation set, the localization in the grid of the four 'rendezvous' sites is randomly chosen (but their positions do not change along the 42 runs of the simulation set). At the start of each run, the presence of the agents depends from their real frequency of occurrence (i.e. the probability of appearance is deduced from field data gathered during 42 evening dives). Therefore, the possible number of agents in the grid for each run ranges between 0 and 11. The spatial distribution of hamlets is always changed at the beginning of each run: 'territorials' are randomly assigned to one of the four 'rendezvous' sites and 'aggressive mimics' are randomly placed in one of the 5041 cells of the grid.

(2) Input data
The model does not use input data to represent time-varying processes.

(3) Submodels
The model is implemented in the R statistical environment (R Core Team 2016).
- The ***move*** submodel is responsible for the spatial dynamics of the 'aggressive mimics' and determines their movement at each time step (every 3.3 seconds). The velocity of the 'aggressive mimics' is constant and corresponds to 30 cm s^{-1}, a value deduced from the average swimming speed in Serranidae (Fisher and Hogan 2007; Fulton 2007) and consistent with what was observed in the field. The 'aggressive mimics' move without any constraint in the whole spawning area (71 m x 71 m). The movement of the 'aggressive mimics' follows random patterns with a queen's move scheme (Figure B1a). In the sensitivity analysis presented below we also implemented alternative simulations by replacing the queen's move

with the rock's move (Figure B1b). 'Aggressive mimics' stop moving until the end of the simulation run once they pair.

- Two 'aggressive mimics' ***pair*** when they are in the same cell. 'Territorials' ***pair*** if they occupy the same 'rendezvous' site. A pair composed of one 'territorial' and one 'aggressive mimic' forms when the latter enters a 'rendezvous' site and finds an unpaired 'territorial'. Two agents that pair are not available for pairing with other agents until the end of the simulation run.

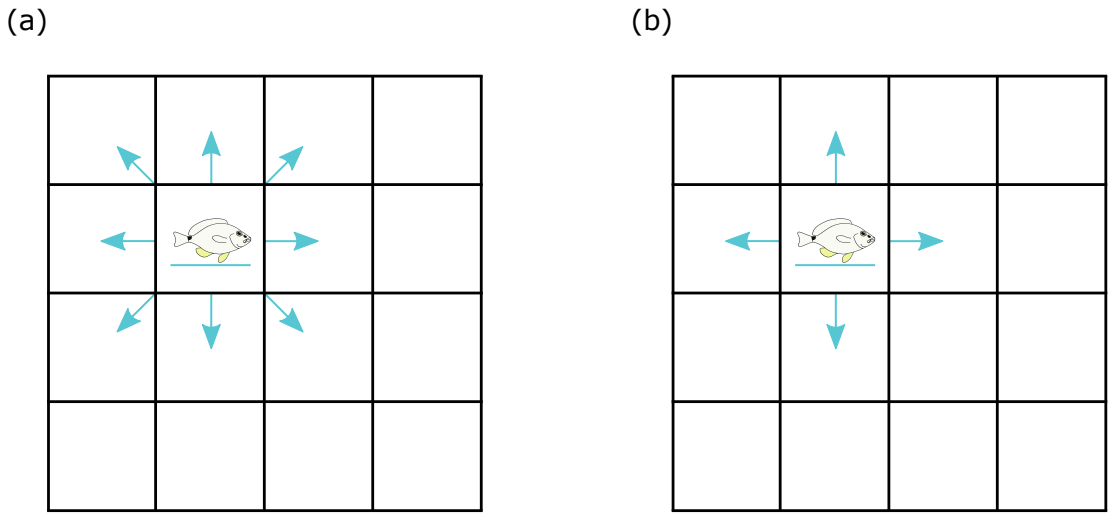


Figure B1. Schemes adopted for modelling the movement of the 'aggressive mimics': (a) queen's move; (b) rock's move. The queen's move is used in the reference model, while the consequences of rock's move on simulation outcomes are investigated in the sensitivity analysis.

Model analysis

The model described in this section was executed for 1000 complete simulation sets. Each set is composed of 42 runs (Figure B2a shows the spatial dynamics of an 'aggressive mimic' during a simulation run). At the end of the simulation set, we extracted: (A) the number of assortative pairings among 'aggressive mimics'; (B) the number of assortative pairings among 'territorials'; and (C) the number of disassortative pairings among 'aggressive mimics' and 'territorials'. After having completed the 1000 complete simulation sets we obtained frequency distributions for assortative and disassortative pairings. We compared the frequency distributions based on the results of simulations with the values from empirical observations (i.e. from the 42 evening dives executed in the field). To this aim, we computed two-tail quantiles (at 1%, 5% and 10% significance levels) for the numbers of assortative and disassortative pairings after 1000 complete sets of simulations. We found that the level of assortative pairing among the 'aggressive mimics' from the field (m = 10) lies outside of the lower and upper limits defined by two-tail quantiles at 5% significance level (Figure B2b). In particular, the empirical level of assortative pairing among the 'aggressive mimics' is larger than the upper threshold defined by the 97.5th percentile. This means that the field

observations allow concluding for a significantly higher number of assortative pairings among ‘aggressive mimics’ than expected from randomness. Empirical values of assortative pairings among ‘territorials’ (Figure B2c) and disassortative pairings (Figure B2d) are included in the 99%, 95% and 90% quantile intervals computed using simulations. Empirical numbers of assortative and disassortative pairings, as well as thresholds for the two-tail quantiles based on simulated data are summarized in Table B1.

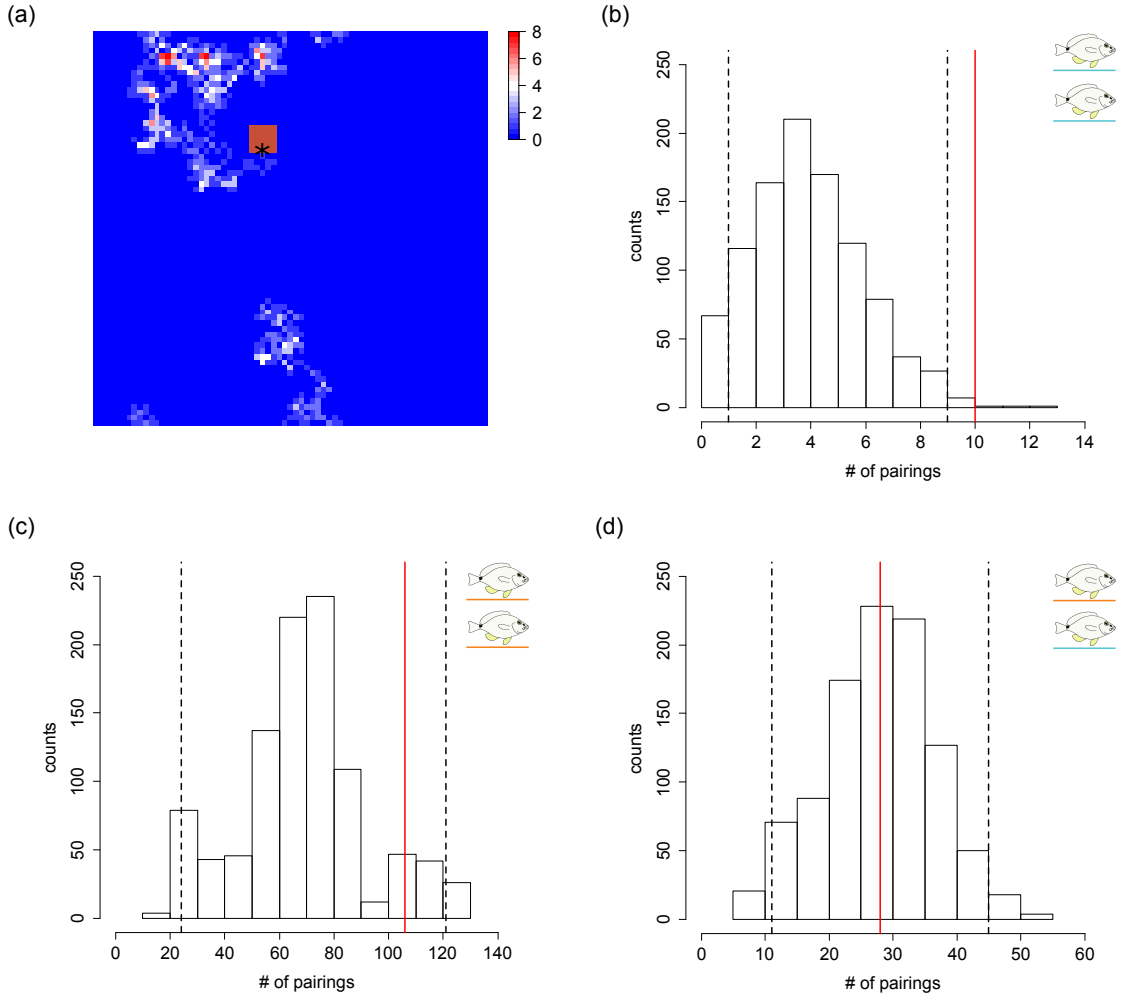


Figure B2. Comparison between numbers of assortative and disassortative pairings obtained with simulations and empirical values from field observations. The random move of an 'aggressive mimic' butter hamlet recorded during a simulation is visualized in (a). The agent moves in the square spawning area by occupying cells of size 1 m x 1 m. The color of the cells reflects the number of times the agent passed through them (ranging between 0 and 8, see scale next to the grid). The 'rendezvous' site where the agent ended and paired with a 'territorial' butter hamlet is highlighted in orange. The cell where pairing occurred is indicated by a black asterisk. The histograms show the frequency distributions (counts) for the number of pairings obtained from simulations. They refer to assortative pairings among 'aggressive mimics' (b), assortative pairings among 'territorials' (c) and disassortative pairings (d). Next to histograms, 'territorials' are illustrated in orange and 'aggressive mimics' in blue. Dotted black lines define the 2.5th and 97.5th percentiles (i.e. 95% of the number of pairings counted in each simulation set falls within these lines). The solid red lines refer to the number of pairings observed in the field in Bocas del Toro, Panama. Only the number of assortative pairings among 'aggressive mimics' from the field lies outside the limits of the 2.5th and 97.5th percentiles (b). In particular, the empirical value is higher than the threshold identified with the 97.5th percentile. This means that the number of assortative pairings among 'aggressive mimics' observed in the field is significantly higher than expected from randomess.

	empirical data	percentiles					
		0.50%	99.50%	2.50%	97.50%	5.00%	95.00%
assortative pairings ('aggressive mimics')	**10**	0	10	1	9	1	8
assortative pairings ('territorials')	**106**	21	127	24	121	27	114
disassortative pairings	**28**	8	49	11	45	13	42

Table B1. Empirical numbers of assortative and disassortative pairings, and limits of quantile intervals for the values obtained with simulations. Thresholds refer to two-tail quantiles at 1%, 5% and 10% significance levels. Empirical numbers of assortative pairings among 'territorial' butter hamlets and disassortative pairings fall within the 5th and 95th percentiles (i.e. empirical data do not deviate from what expected from randomness). A significantly higher number of assortative pairings was observed among 'aggressive mimics' in the field (by considering two-tail quantiles with 5% and 10% significance levels). Grey cells indicate percentiles for which the values observed in the field deviate significantly from the intervals identified using simulated data.

B2. Sensitivity analysis

We carried out a sensitivity analysis to explore to what extent the significantly higher number of pairings among 'aggressive mimics' observed in the field compared to the random expectations generated by the model is a robust outcome. To this aim, we executed alternative simulations by maintaining the same scheme of the reference model (i.e. 1000 simulation sets, each composed of 42 simulation runs). The sensitivity analysis includes models with: (A) different swimming velocities of the 'aggressive mimics'; (B) changes in the move type of the 'aggressive mimics'; (C) 'rendezvous' sites of various sizes; (D) alternative shapes of the simulated spawning area; and (E) different numbers of 'rendezvous' sites. In all cases, we focus on the consequences that such changes in the model have on the number of assortative pairings among 'aggressive mimics'.

(1) Different swimming velocities of the 'aggressive mimics'
In the reference model, the 'aggressive mimics' move with a constant velocity of 30 cm s^{-1}. We executed additional sets of simulations to explore what are the consequences of 'aggressive mimics' moving slower or faster. To this aim, we decreased (or increased) at regular intervals of 10 cm s^{-1} the reference model velocity. This means that we performed new simulations with velocities ranging from 10 cm s^{-1} to 50 cm s^{-1}. All these velocities are realistic and fall into the interval of swimming speeds in Serranidae (Fisher & Hogan 2007, Fulton 2007). For velocities slower than in the reference model, the empirical number of assortative pairings lies above the upper limit of the two-tail quantiles at the 5% significance level (Figure B3a-b), being also significant with the limits of the 0.5th and 99.5th percentiles

(Table B2). When the velocity is 50 cm s^{-1}, the number of assortative pairings in the field falls in the interval determined for simulated data using two-tail quantiles at marginal significance level (Table B2). With the slowest velocity (i.e. 10 cm s^{-1}), the number of disassortative pairings from simulations is significantly lower (5% and 10% significance levels) than what was observed in the field (Table B2).

(2) Changes in the move type of the 'aggressive mimics'

As an alternative to the queen's move adopted in the reference model, we tested the sensitivity of the simulation results to the rock's move (Figure B1b). In this alternative group of simulations, the 'aggressive mimics' can move in four directions only (i.e. diagonal moves are forbidden). We found that in this case the number of assortative pairings observed in the field is still significantly higher than what is expected from randomness (even when considering two-tail quantiles at 1% significance level; see Table B2 and Figure B4).

(3) 'Rendezvous' sites

In the reference model, four spawning 'rendezvous' sites of size 5 m x 5 m are considered. Such areas are randomly placed in the grid at the beginning of each simulation set (i.e. they preserve the same spatial coordinates along a full set of 42 simulation runs). For the sensitivity analysis, we investigated the effect of having progressively smaller 'rendezvous' sites, moving from the reference model configuration (i.e. 25 m^2) to the extreme case of 'rendezvous' sites represented by four single cells only (i.e. 1 m^2). To this aim we progressively decreased of 1 m the size of the 'rendezvous' sites. In all scenarios, 'rendezvous' sites of square shape were considered. With the exception of the 1 m^2 and 4 m^2 'rendezvous' sites, all other scenarios confirmed the significant deviation of empirical values from simulated patterns of assortative pairing among 'aggressive mimics' (Table B2 and Figure B5). Indeed, for 'rendezvous' sites of 9 m^2 and 16 m^2, the empirical value lies above the upper limit found using two-tail quantiles at 5% significance level. In the extreme cases of 1 m^2 and 4 m^2 'rendezvous' sites, the empirical value is marginally significant, being above the upper threshold defined with two-tail quantiles. When 'rendezvous' sites have the smallest size, the number of disassortative pairings observed is significantly higher than expected from simulations (with percentile intervals defined using 5% significance level; see Table B2).

(4) Alternative shape of the spawning area

In the reference model, the spawning area is represented by a 71 m x 71 m square grid. The size of each cell is 1 m x 1 m, and the grid is wrapped up to form a torus to avoid edge effects. As an alternative, we maintained constant all simulation features but changed the shape of the spawning area. In this scenario, the grid has rectangular shape with short dimension = 20 m and long dimension = 250 m (total area = 5000 m^2). These proportions are representative of the spawning area identified in the field. The size of each cell is equal to 1 m x 1 m. Such changes in the shape of the spawning area do not alter the findings of the reference model. Indeed, the empirical number of assortative pairings among the 'aggressive mimics' lies above the upper limit defined by two-tail quantiles at the 5% significance level (Table B2 and Figure B6).

(5) Different number of 'rendezvous' sites

In the reference model we considered four potential spawning 'rendezvous' sites. Since field observations revealed that three of those 'rendezvous' sites were preferentially visited by butter hamlets, we also repeated all previous simulations with three 'rendezvous' sites instead of four. Therefore, in this last group of simulations used for sensitivity analysis we first considered the reference model with three 'rendezvous' sites only. We then carried out other simulations by always changing two properties of the reference model: the number of 'rendezvous' sites (decreasing them to three) and, in turn, any of the parameters discussed in the points from (1) to (4) of this sensitivity analysis section. Histograms comparing the frequency distribution of simulated assortative pairings among 'aggressive mimics' with the empirical value from the field are presented in Figures B7-B11. The histogram in Figure B7 is obtained with a model that corresponds to the reference model except for the number of 'rendezvous' sites (reduced to three). The remaining histograms were obtained with simulations that include three 'rendezvous' sites and investigate changes in: (A) velocities (Figure B8) and move type (Figure B9) of the 'aggressive mimics'; (B) size of the 'rendezvous' sites (Figure B10); and (C) shape of the spawning area (Figure B11). The main outcomes obtained using models that include three 'rendezvous' sites do not deviate from what was observed with four. Only with a velocity of 50 cm s^{-1} there is no significant deviation between the number of assortative pairings involving 'aggressive mimics' in the field and simulations (even with two-tail quantiles at 10% significance level; see Table B3). The number of assortative pairings observed in the field is higher than in the simulations with marginally significant thresholds for 'rendezvous' sites with a size of 9 m^2 or smaller (Table B3). With these latter conditions, the number of disassortative pairings in the field is always above the upper threshold defined with two-tail quantiles at 10% significance level (being even above the upper limit identified with two-tail quantiles at 1% and 5% significance levels in presence of 'rendezvous' sites of either 1 m^2 or 4 m^2; see Table B3).

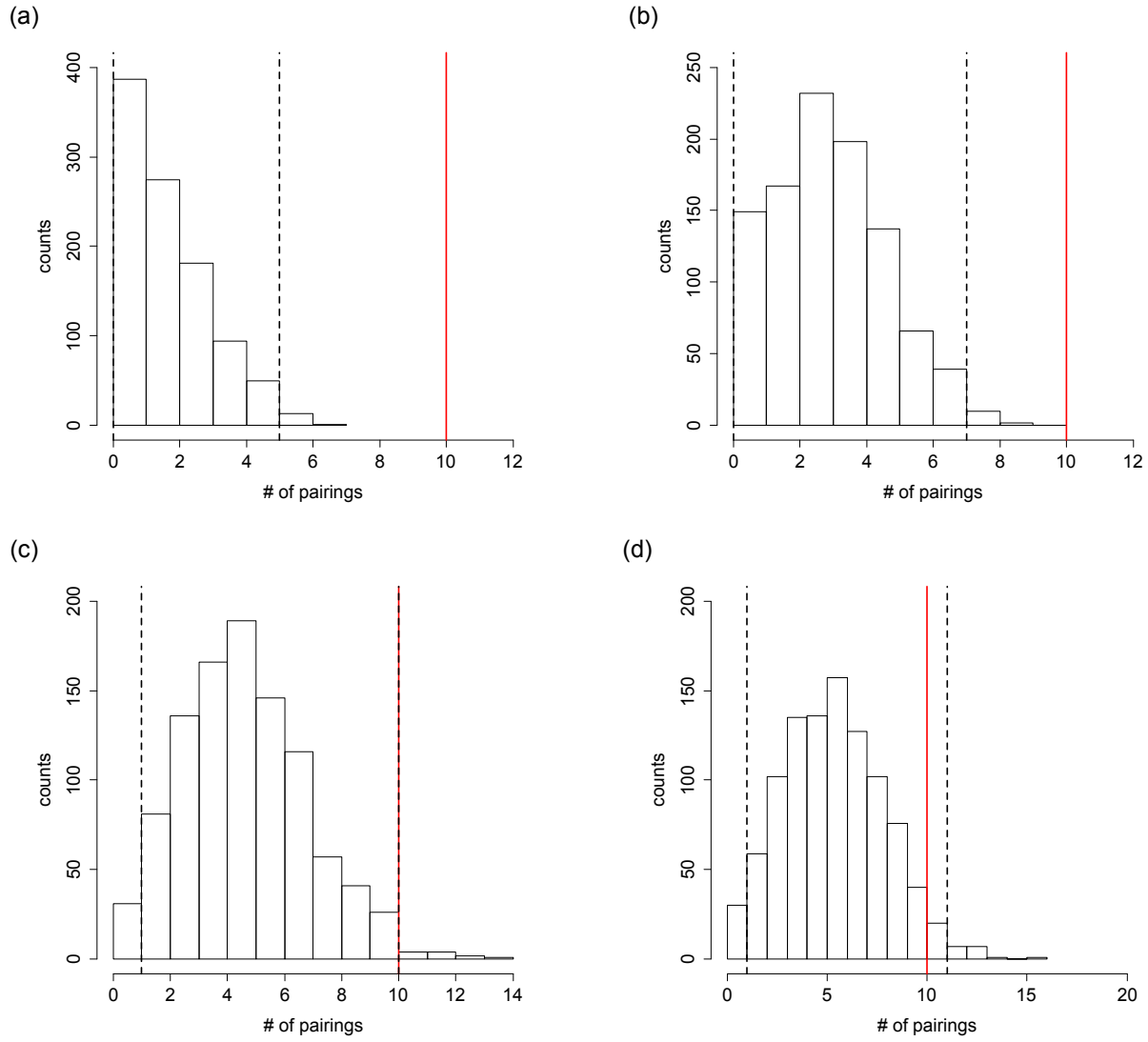


Figure B3. Sensitivity analysis performed by modulating the swimming velocity of the 'aggressive mimic' butter hamlets. The histograms illustrate the frequency distributions of assortative pairing among 'aggressive mimics' in the simulations. In particular, they show the frequency distributions obtained with agents that move with a velocity of 10 cm s^{-1} (a), 20 cm s^{-1} (b), 40 cm s^{-1} (c), or 50 cm s^{-1} (d). The dotted black lines define the limits of the 2.5th and 97.5th percentiles, while the solid red line refers to the number of assortative pairings observed in the field. The number of assortative pairings in the field lies above the values generated from simulations when the 'aggressive mimics' move slower than in the reference model (a-b).

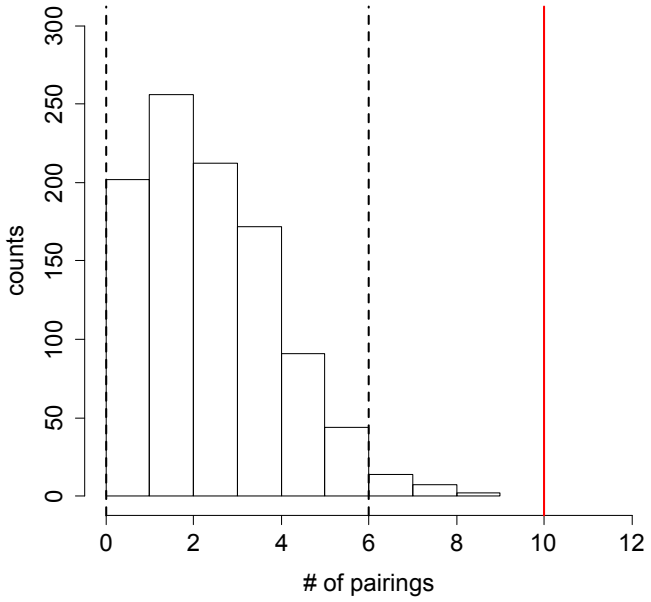


Figure B4. Sensitivity analysis performed by changing the move scheme of 'aggressive mimic' butter hamlets. The frequency distribution of assortative pairings among 'aggressive mimics' is visualized by the histogram. It summarizes the results obtained from simulations where the 2D spatial dynamics of the agents is based on the rock's move. Dotted black lines illustrate the limits of the 2.5th and 97.5th percentiles, and the solid red line indicates the number of assortative pairings observed in the field. This value is significantly higher than what is expected from the random model used for sensitivity analysis.

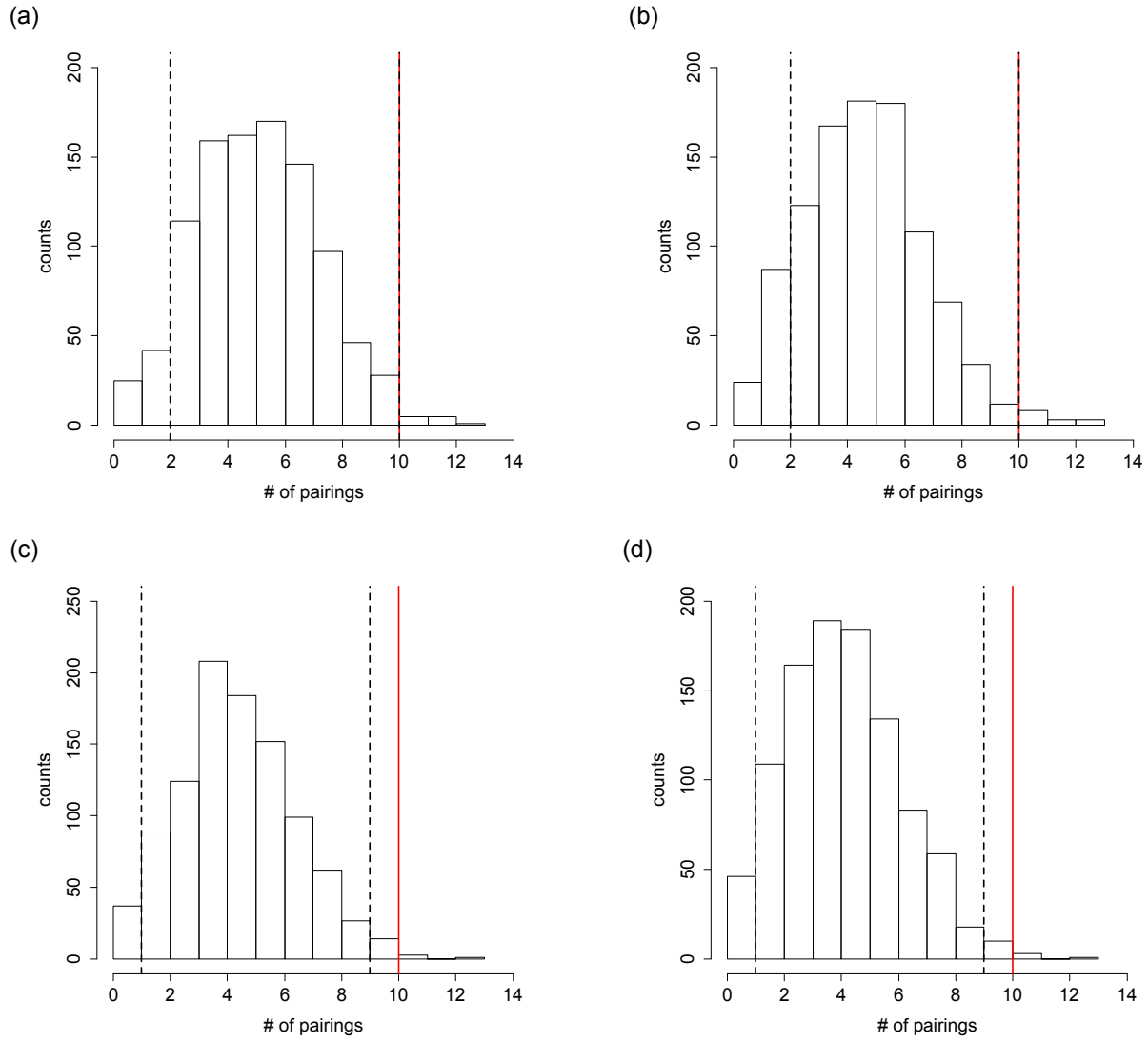


Figure B5. Sensitivity analysis carried out by decreasing the size of the four 'rendezvous' sites. The histograms depict the frequency distribution of assortative pairings among 'aggressive mimics' when the 'rendezvous' sites have sizes of 1 m x 1 m (a), 2 m x 2 m (b), 3 m x 3 m (c), or 4 m x 4 m (d). Dotted black lines represent the thresholds for the 2.5th and 97.5th percentiles, while the solid red line indicates the number of assortative pairings observed in the field. With areas of either 9 m^2 (c) or 16 m^2 (d) the empirical value is significantly higher than expected from random scenarios (the percentile limits are defined with two-tail quantiles at 5% significance level).

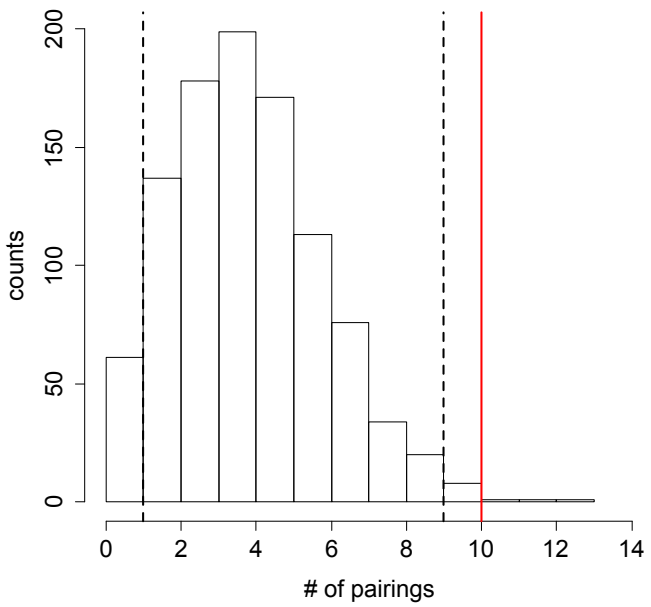


Figure B6. Sensitivity analysis considering simulations performed with a spawning area of rectangular shape. The spawning area was represented by a rectangular grid of size 20 m x 250 m. Frequency distribution of assortative pairing among 'aggressive mimics' from the simulations (histograms) is compared with the benchmark value observed in the field (solid red line). The deviation of the empirical value from randomness is illustrated by the solid red line that lies above the 97.5th percentile of the frequency distribution generated through simulations (see dotted black lines for the limits of the two-tail quantiles at 5% significance level).

	assortative pairing ('aggressive mimics')						assortative pairing ('territorials')						disassortative pairing					
	percentiles																	
	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%
	reference model																	
	0	10	1	9	1	8	21	127	24	121	27	114	8	49	11	45	13	42
	velocity of the 'aggressive mimics'																	
10 cm s^{-1}	0	6	0	5	0	5	20	124	24	119	27	112	3	31	5	27	6	25
20 cm s^{-1}	0	8	0	7	1	7	20	128	24	121	28	116	6	40	8	36	9	34
40 cm s^{-1}	0	12	1	10	2	9	21	130	25	122	28	117	11	56	14	51	16	48
50 cm s^{-1}	1	13	1	11	2	10	21	131	25	122	28	116	11	58	14	54	17	52
	moves of the 'aggressive mimics'																	
rock's move	0	8	0	6	0	6	19	129	25	120	28	113	7	41	9	37	10	34
	size of the 'rendezvous' sites																	
1 m x 1 m	1	12	2	10	2	9	21	125	25	119	27	110	3	28	5	24	6	22
2 m x 2 m	1	12	2	10	2	9	20	128	24	117	27	111	5	36	7	33	9	31
3 m x 3 m	0	10	1	9	2	8	19	126	24	118	27	113	5	42	8	38	10	35
4 m x 4 m	0	10	1	9	2	8	21	130	24	119	27	114	9	47	10	43	12	39
	shape of the spawning area																	
rectangular	0	10	1	9	1	8	21	127	25	119	28	113	6	41	9	37	10	34

Table B2. Threshold values refer to two-tail quantiles for the frequency distributions of assortative and disassortative pairings (with 1%, 5% and 10% significance levels). Such frequencies were obtained by considering the results of simulations performed for sensitivity analysis. Cells have a grey background when the number of pairings observed in the field lies outside the percentile intervals. The number of assortative pairings among 'aggressive mimics' is always larger than expected from randomness (at least, marginally significant), except for the case of agents moving at the highest velocity (i.e. 50 cm s^{-1}). Only simulations with fast fish movements (i.e. 40 cm s^{-1} and 50 cm s^{-1}) and 'rendezvous' sites of small size (i.e. 1 m x 1 m and 2 m x 2 m) generate results with empirical values included in the limits of the 2.5th and 97.5th percentiles. Disassortative pairing appears to occur more frequently in the field than in random models with either slow velocity of 'aggressive mimics' (i.e. 10 cm s^{-1}) or small 'rendezvous' sites (i.e. 1 m x 1 m).

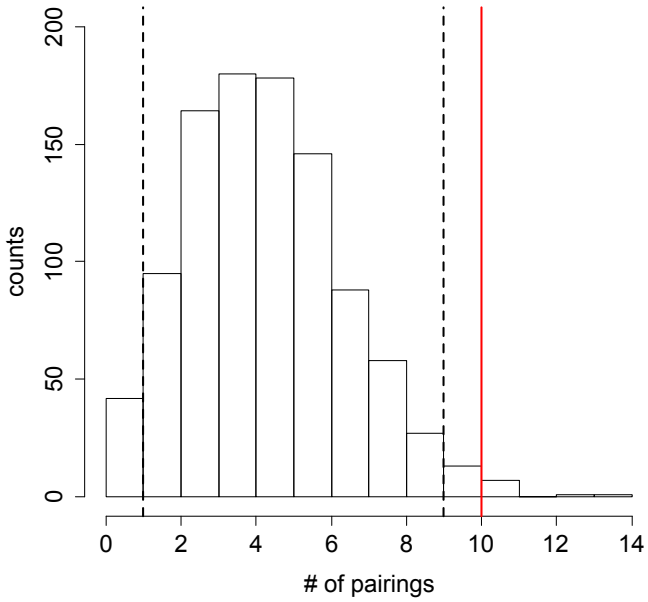


Figure B7. Sensitivity analysis based on a model with three 'rendezvous' sites. All other parameters are the same as in the reference model. The histogram illustrates the frequency distribution of assortative pairing among 'aggressive mimics' as obtained from simulations. Thresholds at the 2.5th and 97.5th percentiles are indicated by dotted black lines. The empirical number of assortative pairings observed in the field (solid red line) is significantly larger than what was obtained from the simulations.

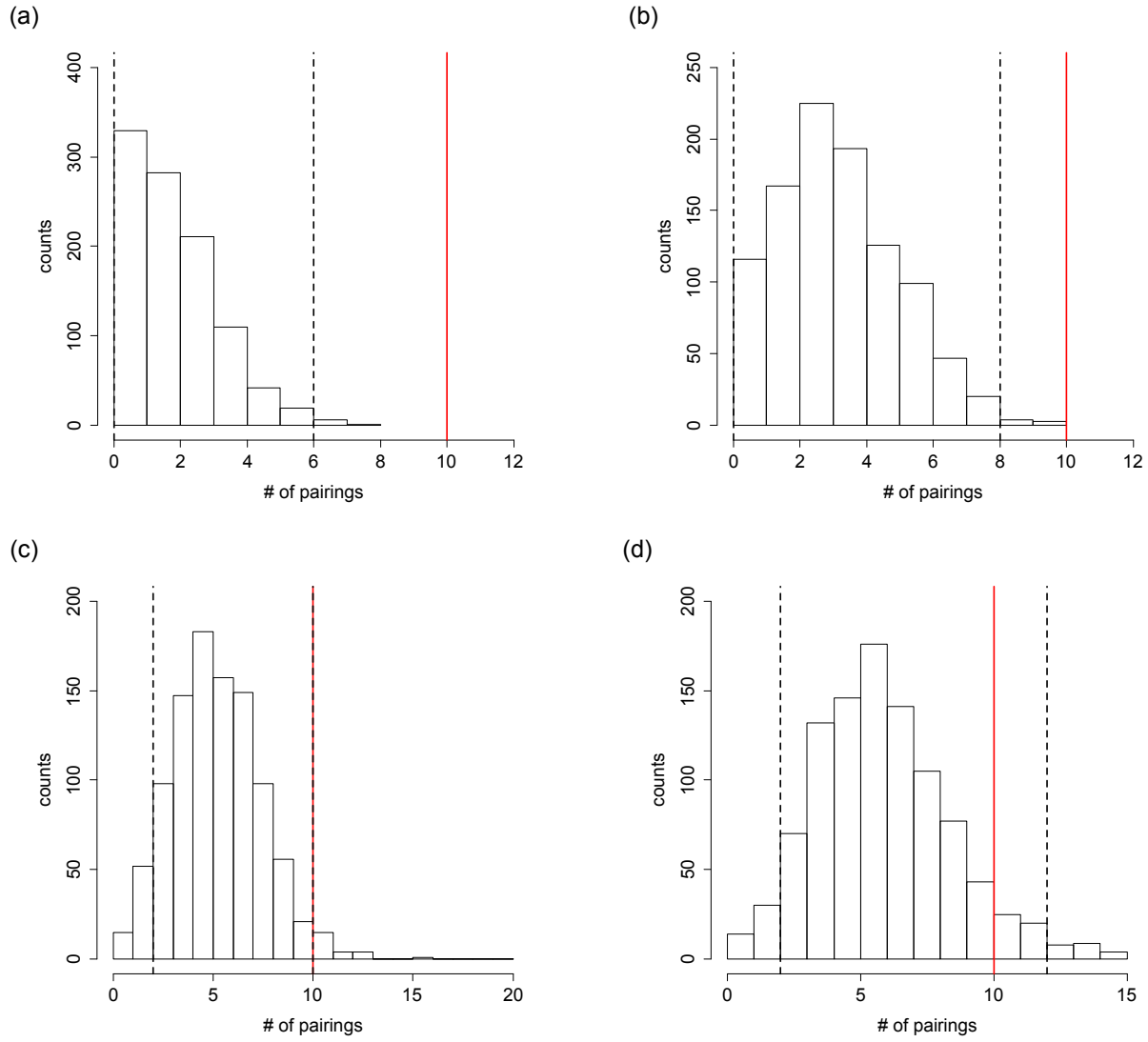


Figure B8. Sensitivity analysis carried out with models that differ from the reference one for the number of 'rendezvous' sites (three) and the velocity of the 'aggressive mimics'. Histograms show the frequency distribution of simulated assortative pairings among 'aggressive mimics' when these have velocities of 10 cm s^{-1} (a), 20 cm s^{-1} (b), 40 cm s^{-1} (c), or 50 cm s^{-1} (d). Limits identified with 2.5th and 97.5th percentiles are delimited by dotted black lines, while the solid red line indicates the empirical number of assortative pairings. Field observations significantly deviate from random frequency distributions when the 'aggressive mimics' swim at relatively slow speeds (a-b).

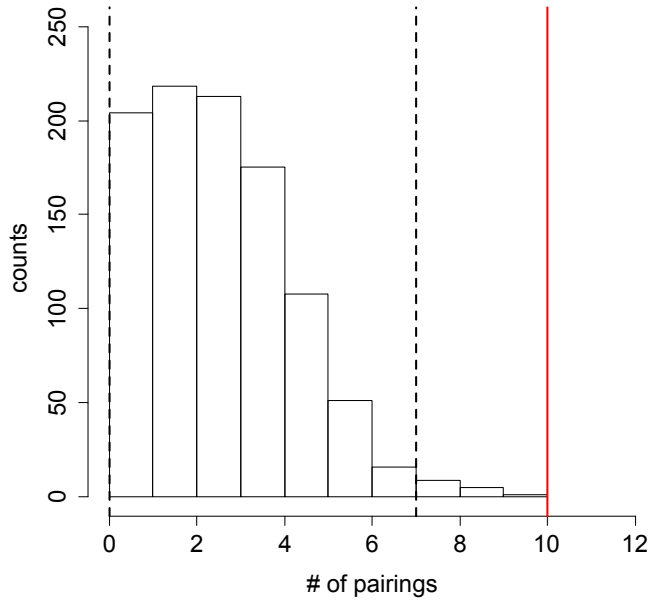


Figure B9. Sensitivity analysis obtained using the model with three 'rendezvous' sites and 'aggressive mimics' moving in four directions only (i.e. rock's move). The histogram shows the frequency distribution of assortative pairing among 'aggressive mimics' as found using simulations. The empirical number of assortative pairings observed in the field (solid red line) is significantly larger than the 97.5th percentile of the distribution based on simulated data. Thresholds for the two-tail quantiles at 5% significance level are visualized with dotted black lines.

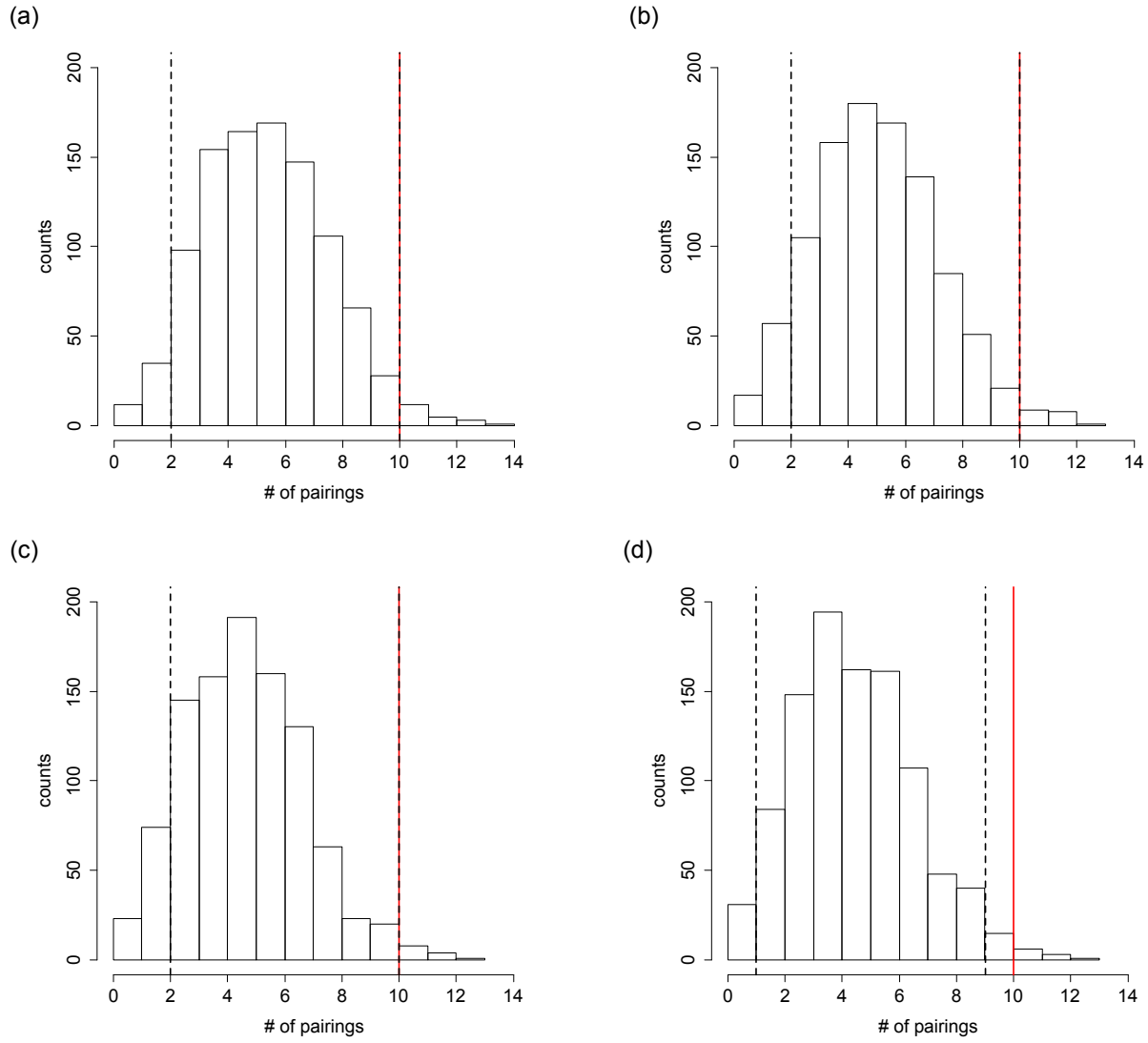


Figure B10. Sensitivity analysis based on models with three 'rendezvous' sites of progressively increasing size. The size of the 'rendezvous' sites is 1 m x 1 m (a), 2 m x 2 m (b), 3 m x 3 m (c), or 4 m x 4 m (d). The histograms illustrate the frequency distributions of simulated assortative pairing among 'aggressive mimics'. Significant thresholds are defined by two-tail quantiles at 5% significance level (dotted black lines) and the number of empirical pairings in the field is denoted by a solid red line. A significantly higher number of field pairings in comparison to random patterns can be detected for areas of 16 m^2 (d). However, in all other cases, the empirical value exceeds the findings of simulations in a marginally significant manner (see Table B3).

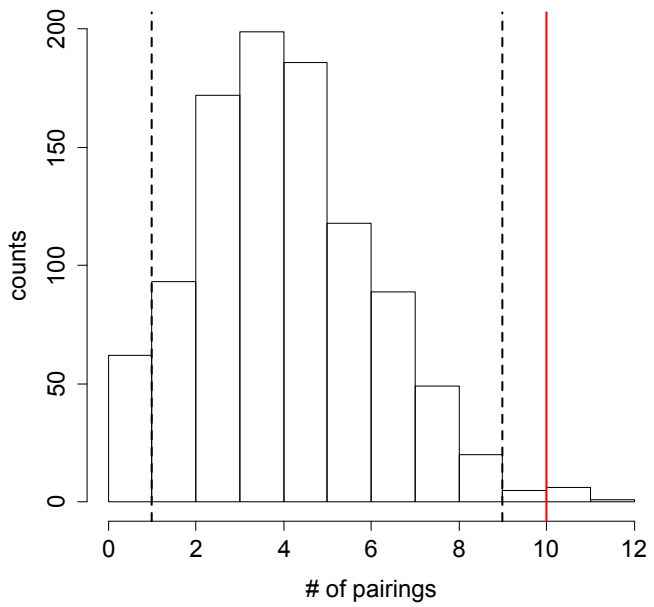


Figure B11. Sensitivity analysis performed with a model with three 'rendezvous' sites in a spawning area of rectangular shape (short dimension = 20 m, long dimension = 250 m). The histogram illustrates the frequency distribution of assortative pairing among 'aggressive mimics' based on simulations. The 2.5th and 97.5th percentiles are delimited by dotted black lines. The number of assortative pairings from the field (solid red line) is significantly different from the frequency distribution generated from the simulations and lies above the upper limit of the two-tail quantiles at 5% significance level.

	assortative pairing ('aggressive mimics')						assortative pairing ('territorials')						disassortative pairing					
	percentiles																	
	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%	0.50%	99.50%	2.50%	97.50%	5.00%	95.00%
	reference model																	
	0	10	1	9	1	8	21	127	24	121	27	114	8	49	11	45	13	42
	number of 'rendezvous' sites																	
three	1	11	1	9	2	8	39	122	43	117	46	113	5	39	8	35	10	33
	swimming velocity of the 'aggressive mimics' (and three 'rendezvous' sites)																	
10 cm s^{-1}	0	7	0	6	0	5	38	124	44	116	46	113	2	22	4	20	5	18
20 cm s^{-1}	0	9	0	8	1	7	37	120	43	116	46	114	5	32	7	29	8	27
40 cm s^{-1}	1	12	2	10	2	9	36	119	42	115	45	112	8	42	11	40	12	38
50 cm s^{-1}	1	14	2	12	3	11	38	122	44	116	47	113	9	47	12	44	14	42
	moves of the 'aggressive mimics' (and three 'rendezvous' sites)																	
rock's move	0	9	0	7	1	6	38	119	43	116	45	112	5	33	7	29	8	28
	size of the three 'rendezvous' sites																	
1 m x 1 m	1	12	2	10	3	9	37	122	44	116	47	113	2	21	3	19	4	18
2 m x 2 m	1	12	2	10	2	9	39	122	42	117	46	113	4	27	5	25	6	24
3 m x 3 m	1	11	2	10	2	9	37	122	42	117	46	113	4	32	6	29	8	27
4 m x 4 m	1	11	1	9	2	9	38	124	44	116	46	112	7	36	9	32	10	31
	shape of the spawning area (and three 'rendezvous' sites)																	
rectangular	0	11	1	9	1	8	37	123	43	117	46	113	4	31	6	29	8	28

Table B3. Limits that define the lower and upper thresholds obtained with two-tail quantiles for the frequency distributions of assortative and disassortative

pairings generated from the simulations (significance levels = 1%, 5% and 10%). Alternative models have been constructed by decreasing the number of 'rendezvous' sites only (i.e. from four to three), or combining this aspect with the ones investigated in the previous group of models for sensitivity analysis (e.g. by both reducing to three the number of 'rendezvous' sites and modulating the swimming velocity of the 'aggressive mimics'). Cells with grey background correspond to scenarios for which a significant deviation (at 1%, 5% or 10% significance level) exists between the number of pairings observed in the field and the number of pairings generated by the simulations. The number of assortative pairings among 'territorials' observed in the field is never significantly different from the values obtained from simulations. The number of disassortative pairings in the field is significantly larger than the simulated values in case of slow swimming speeds of the 'aggressive mimics' (i.e. 10 cm s^{-1} and 20 cm s^{-1}; this latter case holds if a marginal significance level is considered), or with 'rendezvous' sites of smaller size (i.e. with 1 m^2, 4 m^2 and 9 m^2; the 3 m x 3 m case is only marginally significant). The assortative pairing among the 'aggressive mimics' is always significantly different from the patterns generated from simulations (at least when using the interval limits relative to two-tail quantiles at 10% significance level), except for the scenario with agents moving at highest swimming speed. These results confirm the findings of models with four 'rendezvous' sites (Table B2), thus corroborating the robustness of our analysis.

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Figure S1
Number of diurnal 45-minutes observations performed for each fish in 2014 and in 2015.

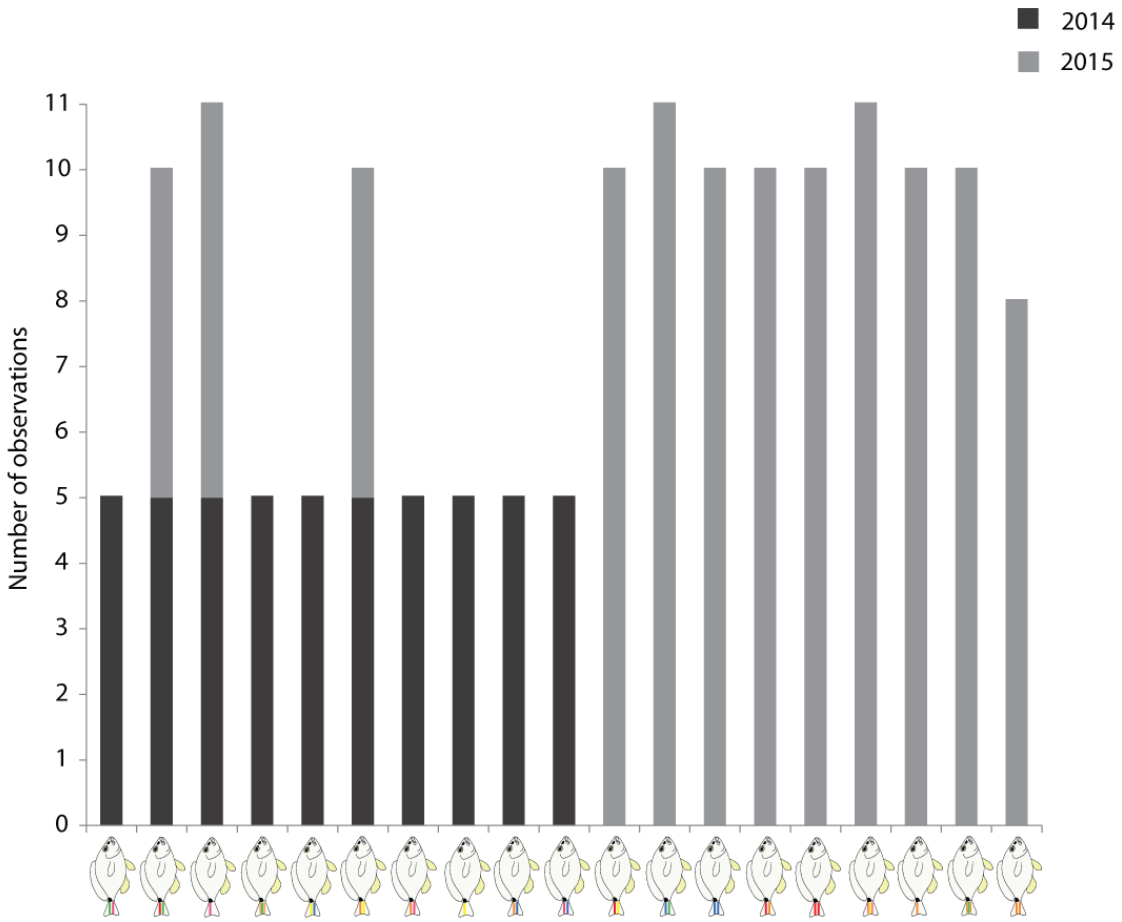


Figure S2
Results of the hierarchical clustering analysis applied to the five behavioral traits listed in Table 1 for 19 butter hamlets (*Hypoplectrus unicolor*) from the Punta Juan reef, Bocas del Toro, Panama. Fish illustrations indicate the identity of each fish, represented by their unique tags on caudal fins. Colors differentiate the two clusters identified: orange corresponds to 'territorials' (n = 13); and blue to 'aggressive mimics' (n = 6).

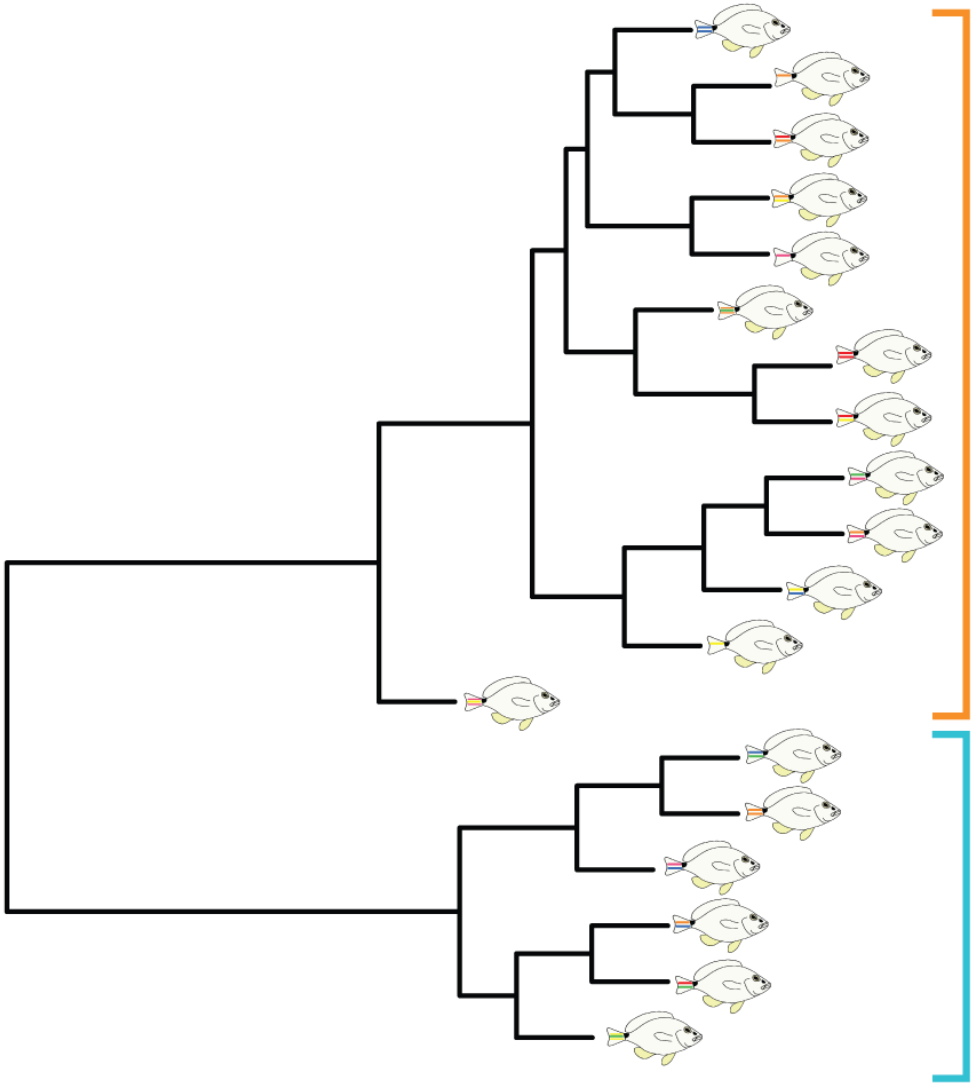


Figure S3

A, Non-metric multidimensional scaling (NMDS) summarizing the two behavioral traits linked to aggressive mimicry, i.e. time spent tracking *C. capistratus* and proportion of foraging bouts performed while tracking *C. capistratus*, for 19 butter hamlets (*Hypoplectrus unicolor*) from the Punta Juan reef, Bocas del Toro, Panama. *B*, Results of hierarchical clustering analysis applied onto these two behavioral traits. Fish illustrations indicate the identity of each fish, represented by their unique tags on caudal fins. The two polygons delineate the two clusters identified by the hierarchical clustering analysis (Figure S3*B*), with orange corresponding to the 'territorials' (n = 13) and blue to the 'aggressive mimics' (n = 6). Fish marked with a star indicate individuals for which diurnal behavioral observations were made in 2015 and for which pairing data was collected as well (see Figure 4*A*).

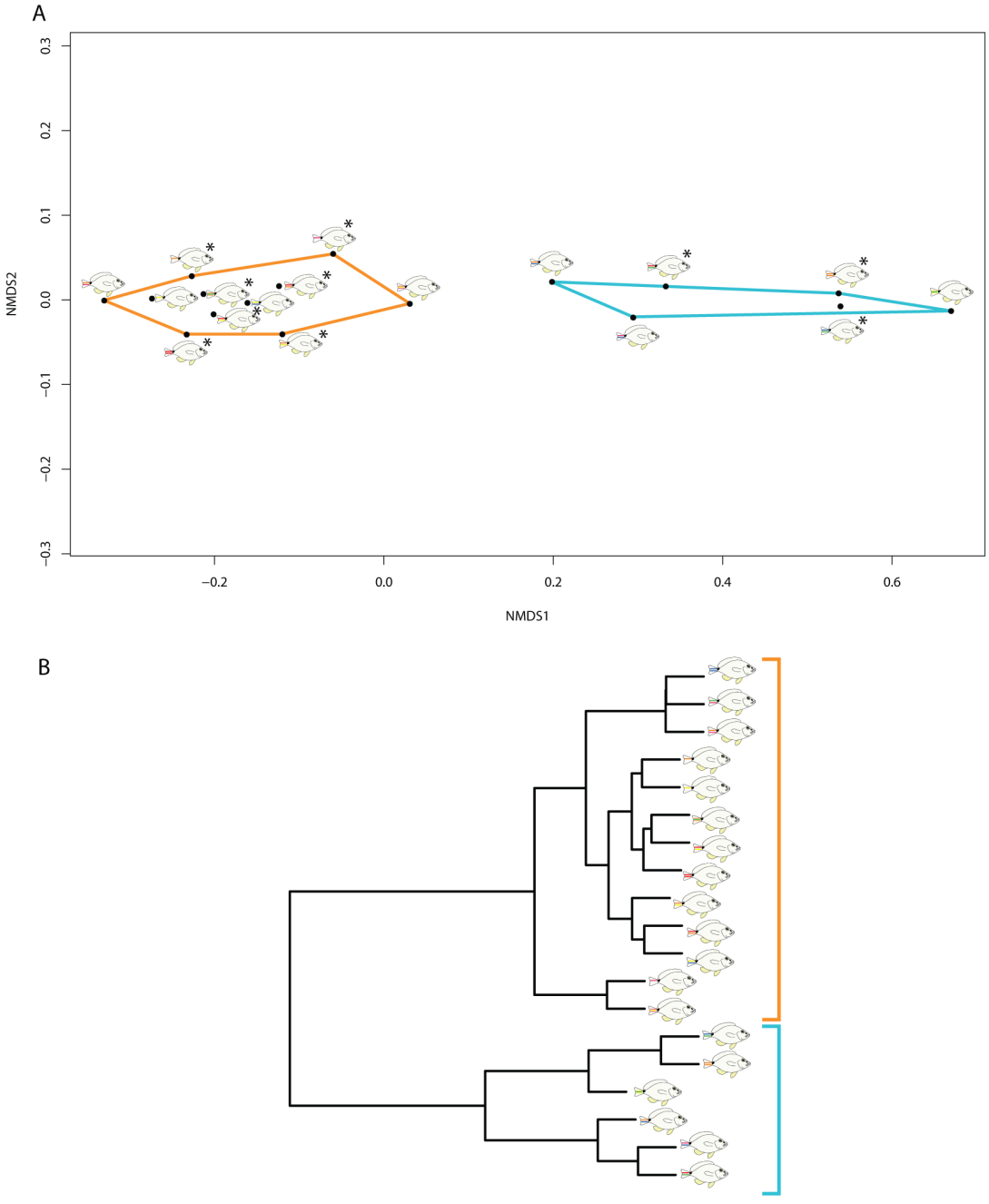


Figure S4
Proportion of foraging bouts performed by the hamlets from the two different behavioral types on eight different food categories or media (note that in the latter case it is not implied that hamlets prey on e.g. sponges but rather that they target small prey on their surface). Proportions were averaged for each fish first and then for each behavioral group. * significant differences at the 0.05 level, derived from multivariate GLMM analysis (see Appendix A1 for details).

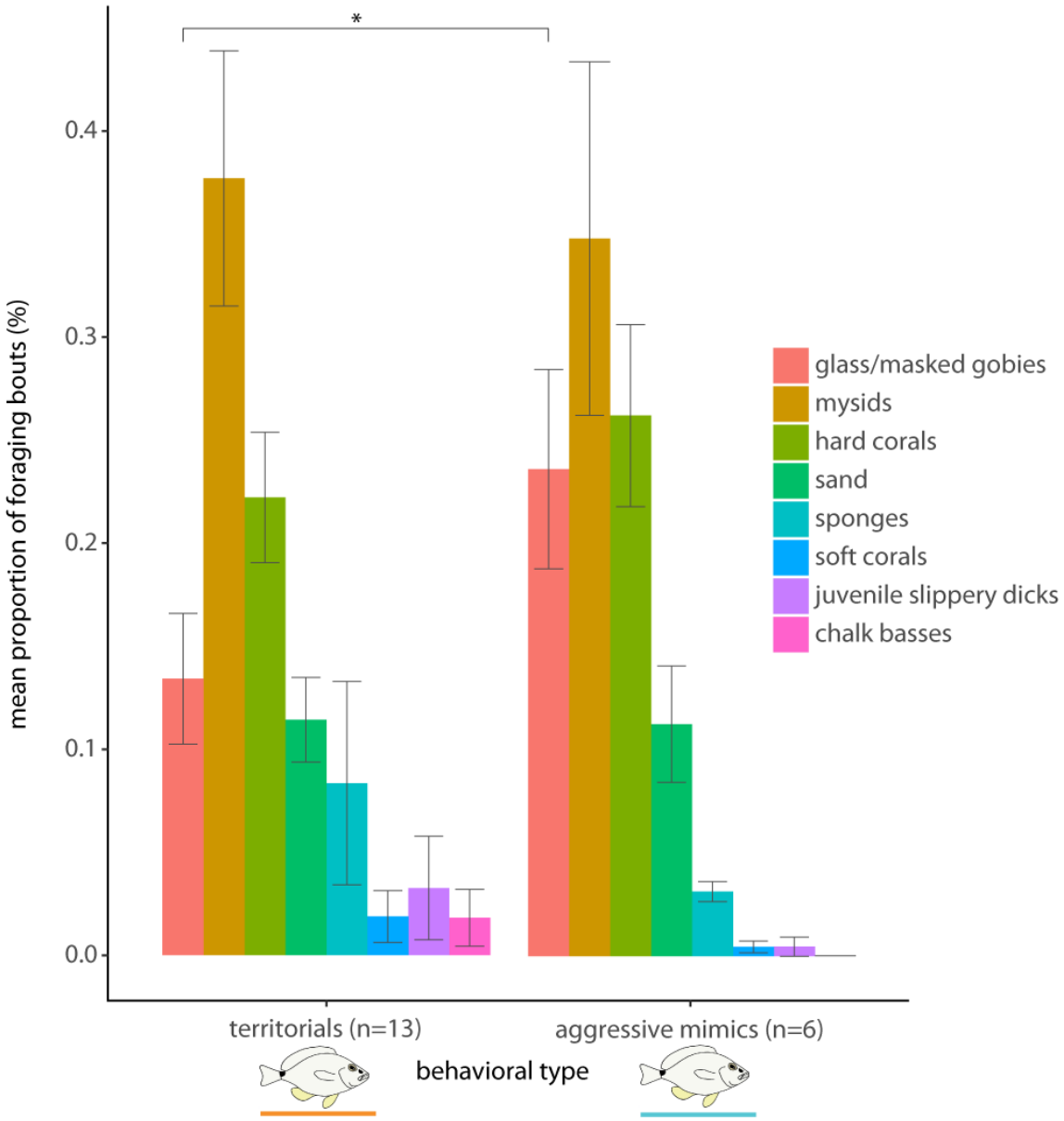


Figure S5
Proportion of foraging bouts performed by the hamlets from the two different behavioral groups on eight different food categories or mediums **while tracking** only (note that in the latter case it is not implied that hamlets prey on e.g. sponges but rather that they target small prey on their surface). Proportions were averaged for each fish first while tracking and then for each behavioral group.

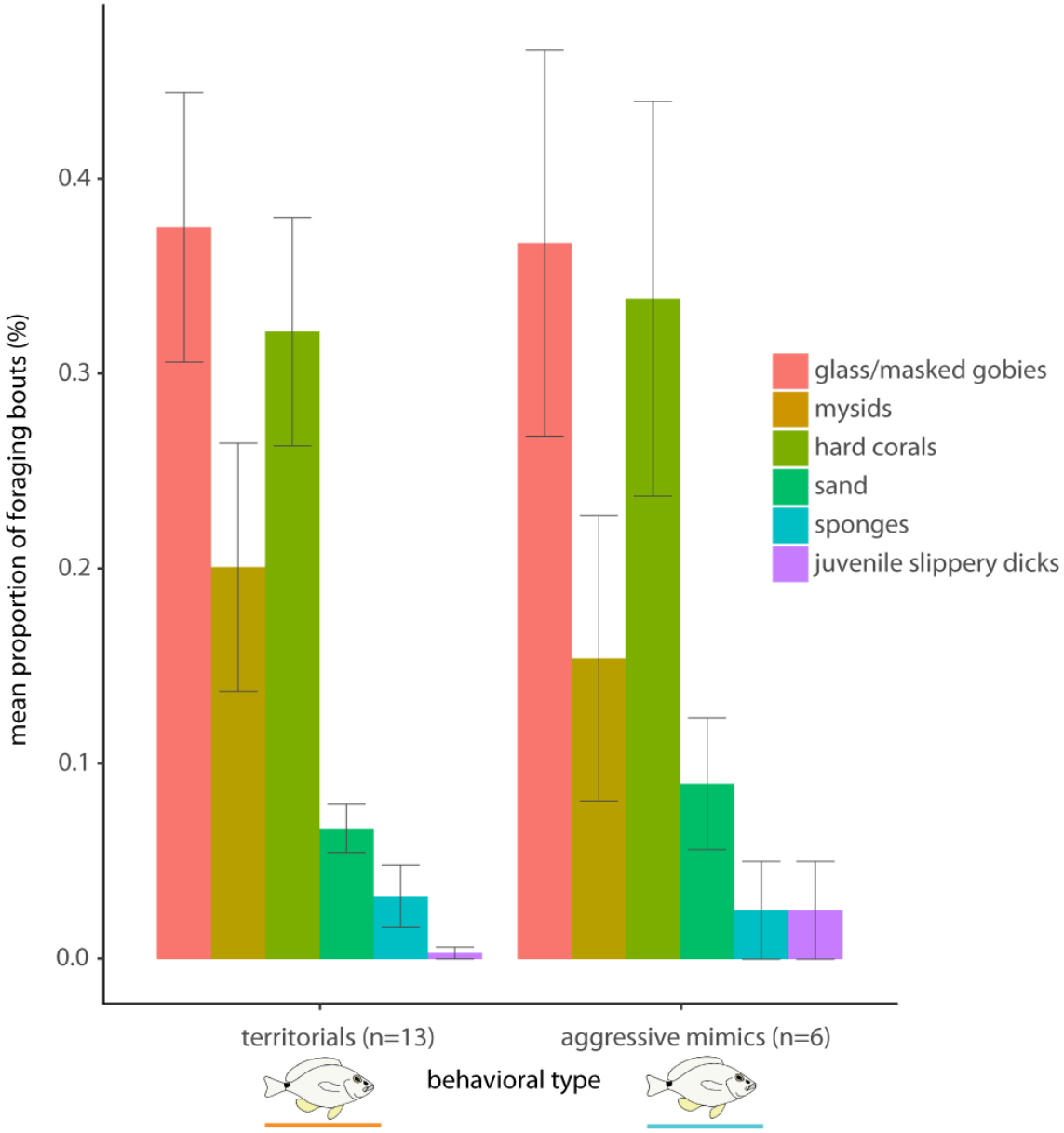
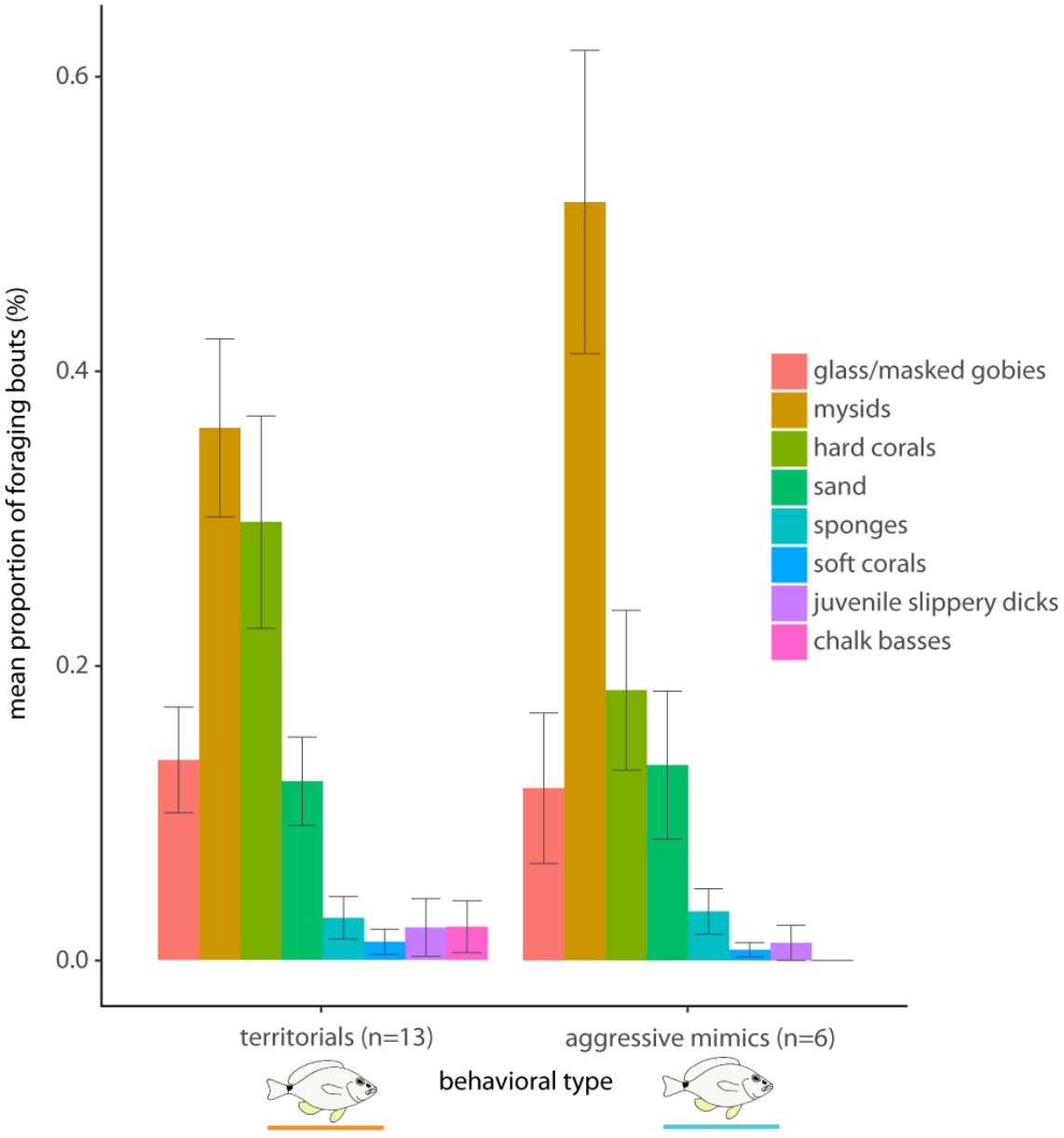


Figure S6
Proportion of foraging bouts performed by the hamlets from the two different behavioral groups on eight different food categories or mediums **while alone** only (note that in the latter case it is not implied that hamlets prey on e.g. sponges but rather that they target small prey on their surface). Proportions were averaged for each fish first while alone and then for each behavioral group.



SUPPORTING INFORMATION FOR CHAPTER III

Appendix S1 Mathematical model – methods

1. Steady-state equations

For each given (*x, y, z*) satisfying (1), steady state requires that for each strategy, the rate of inflow into the egg-carrying state (the left side of the three following equations) balances the

$$x_o = \left(m \frac{x_e + (1-q)y_e + z_e}{e + \lambda o} + \rho \right) x_e, \tag{4a}$$

$$y_o = \rho y_e, \tag{4b}$$

$$z_o = (m + \rho)\, z_e. \tag{4c}$$

outflow from the egg-carrying state (the right side of the three following equations):

On the left side of (4c) we have the proportion of providers not carrying eggs, whose rate of egg-production we have normalized to 1, so that the resulting inflow into the egg-carrying state among providers is simply z_o. Providers carrying eggs lose their eggs at rate ρ due to senescence and at rate m due to meeting other individuals, with each meeting partner willing to accept (*i.e.* fertilize) the eggs offered by a provider. This gives the outflow from the egg-carrying state for providers and the right side of (4c). The explanation for the expressions on the left sides of (4a) and (4b) is analogous. As withholders never give up their eggs when meeting a partner, they only lose eggs due to senescence, explaining the right side of (4b). To understand the right side of (4a), observe that in a meeting with another individual, an egg-carrying trader only gives up its eggs if its partner is also carrying eggs and is not identified as a withholder. Hence, the proportion of meetings in which an egg-carrying trader provides eggs is given by the proportion

of meetings in which this condition is satisfied. As a fraction $e + \lambda o$ of the individuals in the population are available for meetings, this proportion is given by:

$(xe + (1 - q)ye + ze) / (e + \lambda o)$.

Substituting from (2) and (3a), we can rewrite the steady-state equations (4) solely in terms of (x, y, z) and (xe, ye, ze) as:

$$x = \left(m \frac{x_e + (1-q)y_e + z_e}{\lambda + (1-\lambda)(z_e + x_e + y_e)} + \rho + 1 \right) x_e, \tag{5a}$$

$$y = (\rho + 1)\, y_e, \tag{5b}$$

$$z = (m + \rho + 1)\, z_e, \tag{5c}$$

which we can arrange as:

$$\frac{x_e}{x} = \frac{1}{1 + m\frac{x_e + (1-q)y_e + z_e}{e + \lambda o} + \rho}, \tag{6a}$$

$$\frac{y_e}{y} = \frac{1}{1 + \rho}, \tag{6b}$$

$$\frac{z_e}{z} = \frac{1}{1 + m + \rho}, \tag{6c}$$

whenever the population share of the strategy under consideration is strictly positive.

For any $(x, y, z) \in \Delta$, the equations in (5) have a unique solution (x_e, y_e, z_e) satisfying $0 \leq x_e < x$, $0 \leq y_e < y$, and $0 \leq z_e < z$, where the equalities $x_e = 0$, $y_e = 0$, and $z_e = 0$ hold, if and only if the corresponding population share is equal to zero. This claim is immediate for y_e and z_e, for which we have:

$$y_e = \frac{y}{1 + \rho}, \tag{7}$$

$$z_e = \frac{z}{1 + m + \rho}, \tag{8}$$

from (5b) and (5c). Because the term in parenthesis premultiplying x_e in (5a) is strictly positive, it is also immediate that $x_e = 0$ holds if and only if $x = 0$.

2. Male, female, and total reproductive success for each strategy

The expected number of offspring produced via reproduction in both sex roles is the fitness measure. The fitness for the three different strategies are calculated as follows:

Traders

Traders carrying eggs will encounter mates at rate m, but release their eggs only if their partners have eggs themselves and cannot be identified as cheaters. Traders hence gain reproductive success through the female function at rate:

$$w_{\mathrm{T}}^{\mathrm{F}} = \frac{x_e}{x} m \frac{x_e + (1-q)y_e + z_e}{e + \lambda o}.$$

Traders gain reproductive success through the male function *i.* when carrying eggs when they meet providers or traders (at rate m), and *ii.* when not carrying eggs, only when they meet providers (at the lower rate λm). Hence they gain reproductive success through the male function at rate:

$$w_{\mathrm{T}}^{\mathrm{M}} = \frac{x_e}{x} m \frac{x_e + z_e}{e + \lambda o} + \frac{x_o}{x} m \lambda \frac{z_e}{e + \lambda o}.$$

The total fitness of traders is then:

$$
\begin{aligned}
w_{\mathrm{T}} &= w_{\mathrm{T}}^{\mathrm{F}} + w_{\mathrm{T}}^{\mathrm{M}} \\
&= \frac{x_e}{x} m \frac{x_e + (1-q)y_e + z_e}{e + \lambda o} + \frac{x_e}{x} m \frac{x_e + z_e}{e + \lambda o} + \frac{x_o}{x} m\lambda \frac{z_e}{e + \lambda o} \\
&= \frac{x_e}{x} m \frac{2x_e + (1-q)y_e + 2z_e}{e + \lambda o} + \left(1 - \frac{x_e}{x}\right) m\lambda \frac{z_e}{e + \lambda o} \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{x_e}{x} m \frac{2x_e + (1-q)y_e + (2-\lambda)z_e}{e + \lambda o}. \qquad (14)
\end{aligned}
$$

Withholders

Withholders never release eggs. Hence, they gain no reproductive success through the female function: $w_{\mathrm{H}}^{\mathrm{F}} = 0.$

Withholders gain reproductive success through the male function *i.* if carrying eggs, when meeting providers or meeting traders that do not identify them as withholders (at rate *m*), or *ii.* if not carrying eggs, when meeting providers (at the lower rate λm). We then have:

$$
w_{\mathrm{H}}^{\mathrm{M}} = \frac{y_e}{y} m \frac{(1-q)x_e + z_e}{e + \lambda o} + \frac{y_o}{y} m\lambda \frac{z_e}{e + \lambda o}.
$$

Hence, the total fitness to withholders is:

$$
\begin{aligned}
w_{\mathrm{H}} &= w_{\mathrm{H}}^{\mathrm{F}} + w_{\mathrm{H}}^{\mathrm{M}} \\
&= \frac{y_e}{y} m \frac{(1-q)x_e + z_e}{e + \lambda o} + \frac{y_o}{y} m\lambda \frac{z_e}{e + \lambda o} \\
&= \frac{y_e}{y} m \frac{(1-q)x_e + z_e}{e + \lambda o} + \left(1 - \frac{y_e}{y}\right) m\lambda \frac{z_e}{e + \lambda o} \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{y_e}{y} m \frac{(1-q)x_e + (1-\lambda)z_e}{e + \lambda o}. \qquad (15)
\end{aligned}
$$

Providers

Providers carrying eggs encounter mates at rate *m*. Since providers allow any partner to fertilize

their eggs, they gain reproductive success through the female function at rate:

$$w_{\mathrm{P}}^{\mathrm{F}} = \frac{z_e}{z} m.$$

When they carry eggs, providers can also gain male fitness by, again, meeting potential mates at rate *m* and fertilizing their partners' eggs if these are either providers carrying eggs or traders carrying eggs. When they do not carry eggs, providers encounter mates at the lower rate λm and only get to fertilize the eggs of a partner if this partner is another provider carrying eggs. Hence, providers gain reproductive success through the male function at rate:

$$w_{\mathrm{P}}^{\mathrm{M}} = \frac{z_e}{z} m \frac{x_e + z_e}{e + \lambda o} + \frac{z_o}{z} \lambda m \frac{z_e}{e + \lambda o}.$$

The total fitness to providers can then be written as:

$$\begin{aligned}
w_{\mathrm{P}} &= w_{\mathrm{P}}^{\mathrm{F}} + w_{\mathrm{P}}^{\mathrm{M}} \\
&= \frac{z_e}{z} m + \frac{z_e}{z} m \frac{x_e + z_e}{e + \lambda o} + \frac{z_o}{z} m \lambda \frac{z_e}{e + \lambda o} \\
&= \frac{z_e}{z} m \left(1 + \frac{x_e + z_e}{e + \lambda o}\right) + \left(1 - \frac{z_e}{z}\right) m \lambda \frac{z_e}{e + \lambda o} \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{z_e}{z} m \left(1 + \frac{x_e + (1 - \lambda) z_e}{e + \lambda o}\right) \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{z_e}{z} m \left(\frac{e + \lambda o}{e + \lambda o} + \frac{x_e + (1 - \lambda) z_e}{e + \lambda o}\right) \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{z_e}{z} m \left(\frac{\lambda + (1 - \lambda) E}{e + \lambda o} + \frac{x_e + (1 - \lambda) z_e}{e + \lambda o}\right) \\
&= m\lambda \frac{z_e}{e + \lambda o} + \frac{z_e}{z} m \left(\frac{\lambda + (2 - \lambda) x_e + (1 - \lambda) y_e + 2(1 - \lambda) z_e}{e + \lambda o}\right). \qquad (16)
\end{aligned}$$

Appendix S2 Mathematical model – results

S2.1 Pairwise fitness comparisons

Introducing the abbreviations (where the second equality in the first line follows form the definitions in (3, main text)):

$$\alpha = x_e + (1-q)y_e + z_e = e - qy_e, \tag{19a}$$
$$\beta = \lambda + (1-\lambda)e, \tag{19b}$$
$$\gamma = x_e + (1-\lambda)z_e, \tag{19c}$$

We can rewrite equation (18, main text) as

$$w_{\mathrm{T}} = \frac{x_e}{x}(\alpha + \gamma), \tag{20a}$$
$$w_{\mathrm{H}} = \frac{y_e}{y}(\gamma - qx_e), \tag{20b}$$
$$w_{\mathrm{P}} = \frac{z_e}{z}(\beta + \gamma). \tag{20c}$$

Replacing the ratios on the right side of these equations by the expressions in equation (6) and using (from (6a), (19a), and (19b))

$$\frac{x_e}{x} = \frac{\beta}{\beta(1+\rho) + m\alpha},$$

yields

$$w_{\mathrm{T}} = \frac{\beta}{\beta(1+\rho) + m\alpha}(\alpha + \gamma), \tag{21a}$$
$$w_{\mathrm{H}} = \frac{1}{1+\rho}(\gamma - qx_e), \tag{21b}$$
$$w_{\mathrm{P}} = \frac{1}{1+m+\rho}(\beta + \gamma). \tag{21c}$$

Throughout the following we will write $=_s$ to indicate that two expression have the same sign (either +, -, or 0).

Comparison of w_P and w_T

From (21c) and (21a) we obtain that $w_P=w_T$ holds, if and only if $\beta(1+\rho)=m\gamma$:

$$\begin{aligned} w_{\mathrm{P}} = w_{\mathrm{T}} &\Leftrightarrow [\beta(1+\rho)+m\alpha](\beta+\gamma) = \beta(1+m+\rho)(\alpha+\gamma) \\ &\Leftrightarrow [\beta(1+\rho)+m\alpha]\beta + m\alpha\gamma = \beta(1+m+\rho)\alpha + m\beta\gamma \\ &\Leftrightarrow \beta[\beta(1+\rho)-m\gamma] = \alpha[\beta(1+\rho)-m\gamma] \\ &\Leftrightarrow \beta(1+\rho) = m\gamma, \end{aligned}$$

where the last equivalence follows from observing, first, that from (19a) and (19b) we have $\beta - \alpha = \lambda(1-e) + qy_e$ and, second, that the latter expression is strictly positive as we have assumed $\lambda > 0$ and every steady-state satisfies $e < 1$ – unless we have $\rho = 0$ and $y = 1$, in which case the term $qy_e = qy$ is strictly positive as we have assumed $q > 0$. The same line of reasoning holds when we start with inequality rather than equality, showing

$$w_{\mathrm{P}} - w_{\mathrm{T}} =_s \beta(1+\rho) - m\gamma. \tag{22}$$

Comparison of w_P and w_H

Using (21c) and (21b) we obtain

$$\begin{aligned} w_{\mathrm{P}} = w_{\mathrm{H}} &\Leftrightarrow (1+\rho)(\beta+\gamma) = (1+m+\rho)(\gamma - qx_e) \\ &\Leftrightarrow \beta(1+\rho) = m\gamma - (1+m+\rho)qx_e. \end{aligned}$$

Similar reasoning implies that the sign of w_P-w_H coincides with the sign of $\beta(1+\rho) - m\gamma + (1+m+\rho)qx_e$:

$$w_{\mathrm{P}} - w_{\mathrm{H}} =_s \beta(1+\rho) - m\gamma + (1+m+\rho)qx_e. \tag{23}$$

Comparison of w_T and w_H

Using (21a) and (21b) we obtain

$$w_{\mathrm{T}} = w_{\mathrm{H}} \Leftrightarrow (1+\rho)\,\beta(\alpha+\gamma) = [\beta(1+\rho)+m\alpha]\,(\gamma - qx_e)$$
$$\Leftrightarrow \beta(1+\rho)(\alpha+qx_e) = m\alpha\,(\gamma - qx_e)\,.$$

Similar reasoning implies that the sign of $w_T - w_H$ coincides with the sign of $\beta(1+\rho)\,(\alpha + qx_e) - m\alpha\,(\gamma - qx_e)$:

$$w_{\mathrm{T}} - w_{\mathrm{H}} =_s \beta(1+\rho)(\alpha+qx_e) - m\alpha\,(\gamma - qx_e)\,. \qquad (24)$$

S2.2 The replicator dynamics has no interior rest point

If (x, y, z) is an interior rest point of the replicator dynamics, then the associated (x_e, y_e, z_e) satisfies $x_e > 0$, $y_e > 0$, and $z_e > 0$, and we have $w_P = w_T = w_H$. In particular, we must have $w_P = w_T$ and $w_P = w_H$. From (22) and (23) these equalities are equivalent to

$$\beta(1+\rho) = m\gamma \text{ and } \beta(1+\rho) = m\gamma - (1+m+\rho)\,qx_e$$

Substituting the first of these equalities into the second yields $qx_e = 0$. Because q > 0 holds, this contractis $x_e > 0$. Therefore, no interior point exists.

S2.3 Dynamics on the edges

TH-edge

On the TH-edge, the dynamics depend on how λ compares to the critical value

$$\bar{\lambda} = \frac{m(1-q)^2 - (1-q^2)(1+\rho)}{(1+q)(1+\rho)(1+q+2\rho)} \tag{25}$$

in the following way.

1. If $\lambda < \bar{\lambda}$, traders can invade withholders at H, withholders can invade traders at T, and there exists exactly one further rest point $\mathrm{Q} = (x^*, 1-x^*, 0)$ on the TH-edge, where x^* is given by

$$x^* = \frac{-\epsilon + \sqrt{\epsilon^2 - 4\delta\phi}}{2\delta}, \tag{26}$$

with

$$\delta = \frac{q^2(1-\lambda)}{1+\rho}, \tag{27a}$$

$$\epsilon = \frac{m(1-q)^2 - 2q(1+\rho)\left[q + \lambda(1-q+\rho)\right]}{2(1+\rho)^2}, \tag{27b}$$

$$\phi = -\frac{(1-q)\left\{m(1-q) + (1+\rho)\left[1+q+\lambda(1-q+2\rho)\right]\right\}}{4(1+\rho)^2}. \tag{27c}$$

2. If $\lambda \geq \bar{\lambda}$, trading dominates withholding, i.e., the dynamics on the TH-edge are unidirectional, leading from H to T.

To show this, note that on the TH-edge, $z = 0$ and hence $z_e = 0$. Setting $z_e = 0$ in Eq. (18) makes the payoffs for traders and withholders reduce to

$$w_{\mathrm{T}} = \frac{x_e}{x}\left[2x_e + (1-q)y_e\right], \tag{28}$$

$$w_{\mathrm{H}} = \frac{y_e}{y}(1-q)x_e. \tag{29}$$

Replacing the expression for y_e (Eq. (7)) with $y = 1-x$ into the above payoffs and simplifying, we obtain

$$w_{\mathrm{T}} - w_{\mathrm{H}} = \frac{x_e}{(1+\rho)x}\left[2(1+\rho)x_e + (1-q)(1-2x)\right] \tag{30}$$

$$=_s 2(1+\rho)x_e + (1-q)(1-2x), \tag{31}$$

as $x_e/[(1+\rho)x]$ is always positive for $x \in (0,1)$. x_e is uniquely determined by x on the TH-edge by (cf. (5a)))

$$x = \left(m\frac{x_e + (1-q)y_e}{\lambda + (1-\lambda)(x_e+y_e)} + \rho + 1\right)x_e, \tag{32}$$

which can be rearranged as a quadratic in x_e:

$$ax_e^2 + bx_e + c = 0, \tag{33}$$

with

$$a = m + (1+\rho)(1-\lambda), \tag{34a}$$
$$b = m(1-q)y_e + (1+\rho)\left[\lambda + (1-\lambda)y_e\right] - (1-\lambda)x, \tag{34b}$$
$$c = -\left[\lambda + (1-\lambda)y_e\right]x. \tag{34c}$$

Since $a > 0$ and $c < 0$, x_e is given by the unique positive solution to (33), i.e.,

$$x_e = \frac{-b + \sqrt{b^2 - 4ac}}{2a}, \tag{35}$$

where b and c are functions of x.

It follows that the we may view x_e and the expression on the right hand side of (31) as a function of x:

$$f(x) = 2(1+\rho)x_e + (1-q)(1-2x). \tag{36}$$

It is clear that $f(0) = 1 - q > 0$, and that the roots of $f(x)$ satisfy

$$x_e = d, \tag{37}$$

where we have used the abbreviation

$$d = \frac{(1-q)(2x-1)}{2(1+\rho)}. \tag{38}$$

In particular, since $x_e \geq 0$ and $d < 0$ always holds if $x < 1/2$, it must be that roots of $f(x)$ can only exist in the interval $[1/2, 1]$.

Substituting (35) into (37) and performing some algebraic manipulations, we obtain

$$\begin{aligned}
\frac{-b + \sqrt{b^2 - 4ac}}{2a} &= d \\
\sqrt{b^2 - 4ac} &= b + 2ad \\
b^2 - 4ac &= b^2 + 4abd + 4a^2d^2 \\
-c &= bd + ad^2 \\
0 &= g,
\end{aligned}$$

where we defined

$$g = c + bd + ad^2. \tag{39}$$

The roots of $f(x)$ and $g(x)$ coincide. Moreover, since b and d are linear and c is quadratic in x, $g(x)$ is a quadratic function of x that can be rewritten as

$$g(x) = \delta x^2 + \epsilon x + \phi, \tag{40}$$

for real coefficients δ, ϵ, and ϕ. Replacing the expressions for a, b, c (given in Eq. (34)) and the expression for d (given in Eq. (38)), into (39), simplifying, and comparing to (40), we obtain the values of these coefficients, as given by (27). Since $\delta > 0$ and $\phi < 0$ always hold, and by Descartes' rule of signs, $g(x)$ (and hence $f(x)$) has exactly one positive root, at which $g(x)$ changes sign from negative to positive. Let us denote this root by x^*. A necessary and sufficient condition for $x^* < 1$ is that $g(1) > 0$ holds. Calculating $g(1)$ and simplifying, we obtain

$$g(1) = \frac{m(1-q)^2 - (1+q)(1+\rho)\left[1-q+\lambda(1+q+2\rho)\right]}{4(1+\rho)^2}. \tag{41}$$

From this expression, it is immediate that a necessary and sufficient condition for $g(1) > 0$ is that the numerator of (41) is positive, which obtains if and only if $\lambda < \bar{\lambda}$, where $\bar{\lambda}$ is given by (25). In this case, and since $f(0) > 0$, $f(x)$ is positive for $x \in [0, x^*)$ and negative for $(x^*, 1]$. Otherwise, if $\lambda \geq \bar{\lambda}$, $g(1) > 0$ and there is no root of $g(x)$ or $f(x)$ in the interval $(0, 1)$. In this case, it follows that $f(x)$ is positive for all $x \in [0, 1]$.

TP-edge

On the TP-edge, the dynamics depend on how m compares to $1 + \rho$ and on how λ compares to the critical values

$$\lambda_* = \frac{m-(1+\rho)}{\rho(1+\rho)+m(2+\rho)}, \quad \text{and} \quad \lambda^* = \frac{m-(1+\rho)}{(1+\rho)(1+2\rho)}, \tag{42}$$

in the following way:

1. If $m \leq 1+\rho$ or $m > 1+\rho$ and $\lambda^* \leq \lambda$, then providing dominates trading, i.e., the dynamics on the TP-edge are unidirectional, leading from T to P.

2. If $m > 1+\rho$ and $\lambda_* < \lambda < \lambda^*$, there is bistability, i.e., there exists a critical value $x^* \in (0, 1)$ such that $\mathrm{R} = (\hat{x}, 0, 1-\hat{x})$ is a rest point of the replicator dynamics and on the TP-edge the dynamics lead to P for $x < \hat{x}$ and to T for $x > \hat{x}$. This critical proportion of traders is given by

$$\hat{x} = \frac{\zeta - \eta\sqrt{\theta}}{\iota}, \tag{43}$$

where

$$\begin{aligned}
\zeta &= m^3 - \left[2+\rho-\lambda(5+4\rho)\right] m^2 - (1+\rho)\left\{1+\rho+\lambda\left[\lambda(4+\rho)-(7+4\rho)\right]\right\} m \\
&\quad + (1-\lambda)(1+\rho)^2(2+\rho+\lambda\rho) && \text{(44a)} \\
\eta &= (1+m+\rho)\left[m-(1-\lambda)(1+\rho)\right] && \text{(44b)} \\
\theta &= m^2 + \left[\lambda(8+6\rho)-4-2\rho\right] m + (2+\rho+\lambda\rho)^2 && \text{(44c)} \\
\iota &= 4\lambda(1-\lambda)m(1+\rho) && \text{(44d)}
\end{aligned}$$

3. If $m > 1 + \rho$ and $\lambda \leq \lambda_*$, then T dominates P, i.e., the dynamics on the TP-edge are unidirectional, leading from P to T.

To show the above claims, note that, as indicated in (22), the sign of the payoff difference $w_{\mathrm{P}} - w_{\mathrm{T}}$ coincides with the sign of $\beta(1+\rho) - m\gamma$. On the TP-edge, $y = 0$ and hence $y_e = 0$ and $e = x_e + z_e$. Replacing the expressions for β and γ from their definitions (19b) – (19c) we thus obtain

$$\begin{aligned} w_{\mathrm{P}} - w_{\mathrm{T}} &=_s (\lambda + (1-\lambda)(z_e + x_e))\,(1+\rho) - m\,[(1-\lambda)z_e + x_e] \\ &= \lambda\,(1+\rho - m x_e) + (1-\lambda)(z_e + x_e)\,(1+\rho-m)\,. \end{aligned} \tag{45}$$

As both x_e and z_e are uniquely determined by x on the TP-edge, the latter explicitly as

$$z_e = \frac{1-T}{1+m+\rho} \tag{46}$$

(by (5c) and $z = 1-x$) and the former by the unique solution to the equation (cf. (5a)))

$$x = \left(m \frac{x_e + z_e}{\lambda + (1-\lambda)(z_e + x_e)} + \rho + 1 \right) x_e, \tag{47}$$

we may view the expression in (45) as a function of x:

$$h(x) = \lambda\,(1+\rho - m x_e) + (1-\lambda)(z_e + x_e)\,(1+\rho-m) \tag{48}$$

defined on the domain $x \in [0,1]$.

For $m \leq 1+\rho$ the expression on the right side of (48) is clearly strictly positive, so that we obtain $h(x) > 0$ and hence $w_{\mathrm{P}} - w_{\mathrm{T}} > 0$ for all $x \in [0,1]$, establishing that for $m \leq 1+\rho$ providers dominate traders.

Consider $m > 1+\rho$ for the remainder of the argument.

For $x = 0$ we have $x_e = 0$ and $e = z_e = 1/(1+\rho+m)$. Therefore,

$$h(0) =_s (1+m+\rho)\lambda(1+\rho) + (1-\lambda)(1+\rho-m).$$

Consequently, the sign of $h(0)$ coincides with the sign of $\lambda - \lambda_*$ where λ_* is given by Eq. (42).

The derivative of $h(x)$ with respect to x is given by

$$\frac{dh}{dx} = -\lambda m \frac{dx_e}{dx} + (1-\lambda)\,(1+\rho-m)\,\frac{d(x_e+z_e)}{dx}, \tag{49}$$

which (using the inequality $m > 1+\rho$) is strictly negative, provided that both derivatives appearing on the right side of (49) are strictly positive. To show that this is the case, we differentiate both sides of the identity (47) with respect to x to obtain

$$1 = \frac{dx_e}{dx}\,[1+\rho+Am] + m x_e \frac{dA}{d(x_e+z_e)} \frac{d(x_e+z_e)}{dx}, \tag{50}$$

where we have used the abbreviation $A = (x_e+z_e)/(\lambda+(1-\lambda)(x_e+z_e))$. A straightforward calculation verifies that we have $dA/d(x_e+z_e) > 0$. As we also have $dz_e/dx < 0$ and $A > 0$, it follows that $dx_e/dx > 0$ holds. It remains to exclude the possibility that $d(x_e+z_e)/dx \leq 0$. Towards this end, we observe that if $d(x_e+z_e)/dx \leq 0$ holds, then (50) implies $dx_e/dx \geq 1/(1+\rho+Am)$. As $A < 1$ holds, we also have $1/(1+\rho+Am) > 1/(1+\rho+m)$, so that $dx_e/dx > 1/(1+\rho+m)$. As $dz_e/dx = -1/(1+\rho+m)$ it then follows that $d(x_e+z_e)/dx > 0$ holds, yielding a contradiction.

The preceding arguments establish that for $\lambda \leq \lambda^*$ trading is dominant on the TP-edge as $h(x) < 0$ holds for $x > 0$. For $\lambda > \lambda^*$ we have $h(0) > 0$ and $h(x)$ is strictly decreasing. Therefore, if $h(1) \geq 0$ holds, then providing is dominant on the TP-edge. Otherwise, i. e. , if $h(1) < 0$ holds, then there exists a unique value $0 < x^* < 1$ such that $h(x^*) = 0$ holds and there is bistability on the TP-edge with the restpoint corresponding to x^* separating the basins of attraction of T and P.

Consider the condition for $h(1) \geq 0$, ensuring that providing is dominant along the TP-edge. As $x = 1$ implies $z_e = 0$, we have

$$h(1) = \lambda\,(1+\rho-mx_e) + (1-\lambda)x_e\,(1+\rho-m) \tag{51}$$

from (48), and from (47) we have

$$\lambda + (1-\lambda)x_e = (mx_e + (\rho+1)\,[\lambda+(1-\lambda)x_e])\,x_e, \tag{52}$$

and hence,

$$x_e = \frac{1-\lambda(2+\rho)+\sqrt{4\lambda m+(1+\lambda\rho)^2}}{2m+(1-\lambda)(1+\rho)} \tag{53}$$

is the unique positive solution to the quadratic implicitly defined by (52). From Eq. (51), and noting that $m > (1+\rho)(1-\lambda)$ holds (since we assumed that $m > 1+\rho$ holds), the condition $h(1) \geq 0$ can be then written as

$$x_e \leq \frac{\lambda(1+\rho)}{m-(1+\rho)(1-\lambda)}.$$

Substituting Eq. (53) into the above expression, rearranging, and simplifying, we obtain that $h(1) \geq 0$ is equivalent to

$$\sqrt{4\lambda m+(1+\lambda\rho)^2}\,(m-(1+\rho)(1-\lambda)) \leq B, \tag{54}$$

where we have defined

$$B = (1-\lambda)(1+\rho)(1+\lambda\rho) + m\,[\lambda(4+3\rho)-1]\,. \tag{55}$$

The expression on the left hand side of (54) is positive. B can be either negative or nonnegative, depending on parameter values. If it is negative, condition (54) cannot hold, and hence $h(1) < 0$. If it is nonnegative, taking squares of both sides of (54) and simplifying shows that (54) (and hence $h(1) \geq 0$) is equivalent to $\lambda \geq \lambda^*$, where λ^* is given by Eq. (42).

To proceed, we need to consider which of these cases arises under any possible parameter constellation. Towards this end, notice that a necessary and sufficient condition for B to be negative is that both $\lambda < \hat{\lambda}$ and $m > \hat{m}$ hold, where

$$\hat{\lambda} = \frac{1}{4+3\rho}, \tag{56}$$

and

$$\hat{m} = \frac{(1-\lambda)(1+\rho)(1+\lambda\rho)}{1-\lambda(4+3\rho)}. \tag{57}$$

Furthermore, and from equations (42) and (56), the signs of pairwise differences between $\hat{\lambda}$ and the critical values λ_*, λ^* satisfy

$$\hat{\lambda} - \lambda_* =_s m^* - m, \tag{58a}$$

$$\hat{\lambda} - \lambda^* =_s m_* - m, \tag{58b}$$

where m_* and m^* are defined as

$$m_* = \frac{5(1+\rho)^2}{4+3\rho}, \quad \text{and} \quad m^* = 2(1+\rho). \tag{59}$$

Noting that $\lambda_* < \lambda^*$, $m_* < m^*$, and $m_* < \hat{m}$ hold in the relevant intervals, we obtain the following exhaustive list of possible cases.

1. If $1 + \rho < m < m_*$, then $\lambda_* < \lambda^* < \hat{\lambda}$ and $m < \hat{m}$ hold. In this case, B is nonnegative, as $\lambda < \hat{\lambda}$ and $m > \hat{m}$ cannot simultaneously hold. It follows that if $\lambda_* < \lambda < \lambda^*$ then $h(1) < 0$, and if $\lambda \geq \lambda^*$, then $h(1) \geq 0$.

2. If $m_* < m < m^*$, then $\lambda_* < \hat{\lambda} < \lambda^*$ holds. In this case, if $\lambda > \lambda^*$, B is nonnegative, and $h(1) \geq 0$ holds. If $\lambda^* < \lambda < \hat{\lambda}$, then irrespective of B being negative or nonnegative, $h(1) < 0$ holds, since $\lambda < \lambda^*$.

3. If $m^* < m$, then $\hat{\lambda} < \lambda_* < \lambda^*$ holds. In this case, $\lambda > \hat{\lambda}$ holds for all $\lambda > \lambda_*$. Hence, B is nonnegative in the relevant interval. It follows that if $\lambda_* < \lambda < \lambda^*$ then $h(1) < 0$ holds, and if $\lambda > \lambda^*$, then $h(1) \geq 0$ holds.

To find the value $x^* \in (0,1)$ such that $h(x^*) = 0$ holds when there is bistability, we first rewrite (48) explicitly as a function of x. To do so, we solve Eq. for x_e and substitute its positive solution together with the expression for z_e (46) into the expression for $h(x)$ in (48). Noting that x^* is given by the unique solution in the interval $(0,1)$ of the resulting quadratic equation $h(x) = 0$, we can finally obtain the expression for x^* given in Eq. (43)

HP-edge

On the HP-edge, the dynamics depend on how λ compares to the critical value λ_* given in Eq. (42) in the following way.

1. If $\lambda < \lambda_*$, providers can invade at H, withholders can invade at P, and there exists exactly one further rest point $\mathrm{S} = (0, 1 - z^*, z^*)$ on the HP-edge at which the frequency of providers is given by

$$z^* = \frac{(1 + \lambda\rho)(1 + m + \rho)}{2m(1 - \lambda)}. \tag{60}$$

2. If $\lambda \geq \lambda_*$, providers dominate withholders, i.e., the dynamics on the HP-edge are unidirectional, leading from H to P.

To show this, note that on the HP-edge, $x = 0$ and hence $x_e = 0$. Therefore, as indicated in (23), the sign of the payoff difference $w_\mathrm{P} - w_\mathrm{H}$ coincides with the sign of $\beta(1 + \rho) - m\gamma$. Replacing the expressions for β and γ from their definitions (19b) – (19c) and using $e = y_e + z_e$ we thus obtain

$$w_\mathrm{P} - w_\mathrm{H} =_s (\lambda + (1 - \lambda)(y_e + z_e))(1 + \rho) - m(1 - \lambda)z_e.$$

Replacing the expressions for z_e and y_e (Eq. (8) and (7)) with $y = 1 - z$, and simplifying, we obtain

$$w_\mathrm{P} - w_\mathrm{H} =_s n(z), \tag{61}$$

where

$$n(z) = (1 + \lambda\rho)(1 + m + \rho) - 2m(1 - \lambda)z, \tag{62}$$

which is a decreasing linear function of z. Since $n(0) = (1 + \lambda\rho)(1 + m + \rho)$ is positive, $n(z)$ (and hence the payoff difference (61)) is either positive for all $z \in [0, 1]$, or has a single sign change from positive to negative at some $z^* \in (0, 1)$ on the HP-edge.

To check which of these scenarios arises, note that a necessary and sufficient condition for $n(z)$ to change sign from positive to negative is that $n(1) < 0$ holds. This condition is satisfied if and only if $\lambda < \lambda_*$. In this case, the point z^* at which the direction of selection changes is found by solving the equation $n(z^*) = 0$ for z^*. If $\lambda \geq \lambda_*$, $n(1) \geq 0$ and the sign of $n(z)$ (and hence of the payoff difference (61)) is positive in the relevant interval.

S2. 4 Stability analysis of the non-trivial rest points

The previous analysis has identified three non-trivial rest points located in the edges of the simplex: Q (located on the TH-edge), R (located on the TP-edge), and S (located on the HP-edge). Here, we discuss the local stability of these rest points.

Q is a sink

Consider first the rest point Q, located on the TH-edge. From the previous analysis, this rest point is stable in the direction of the TH-edge as it is attracting from both

T and H. Moreover, Q is also attracting for neighboring points in the interior of the simplex. Hence, that Q is a sink.

To show this, note that at Q the fitnesses of traders and withholders are equal, i.e., $w_{\mathrm{T}} = w_{\mathrm{H}}$ holds. By (24) this implies

$$\alpha\left[\beta(1+\rho) - m\gamma\right] + \left[\beta(1+\rho) + m\alpha\right] q x_e = 0. \tag{63}$$

Since at Q we also have $x > 0$ and hence $x_e > 0$, α and β (as defined in) are positive. This implies that, in order for (63) to hold, we require $\beta(1+\rho) - m\gamma < 0$. But, by (22), this is the condition for $w_{\mathrm{P}} < w_{\mathrm{T}}$ to hold. We then have that, at Q, the fitnesses of the three strategies satisfy $w_{\mathrm{T}} = w_{\mathrm{H}} > w_{\mathrm{P}}$, establishing our claim.

R is a saddle

Consider now the rest point R, located on the TP-edge. From the previous analysis, this rest point is unstable in the direction of the TP-edge as it is repelling from both T and P. Moreover, R is attracting for neighboring points in the interior of the simplex. Hence, that R is a saddle.

To show this, note that at R the fitnesses of traders and providers are equal, i.e., $w_{\mathrm{T}} = w_{\mathrm{P}}$ holds. Hence, by (22), $\beta(1+\rho) - m\gamma = 0$. Substituting this identity into (23), and since x_e (as, at R, $x > 0$ holds), we obtain $w_{\mathrm{P}} > w_{\mathrm{H}}$. Hence, at R, the fitnesses of the three strategies satisfy $w_{\mathrm{T}} = w_{\mathrm{P}} > w_{\mathrm{H}}$, establishing our claim.

S is a saddle

Finally, consider the rest point S, located on the HP-edge. From the previous analysis, this rest point is stable in the direction of the HP-edge as it is attracting from both H and P. Moreover, S is repelling for neighboring points in the interior of the simplex. Hence, that S is a saddle.

By similar arguments as above, we get $w_{\mathrm{T}} = w_{\mathrm{H}} = w_{\mathrm{P}}$ at S. We hence need different arguments to show our result.

S2.5 Dynamical regions

Preliminaries

To identify the different dynamical regions, it is useful to consider the critical values of λ on which the existence of the non-trivial points depend, i.e., $\bar{\lambda}$, λ_*, and λ^* (given by equations (25) and (42)) as functions of m. We then write

$$\bar{\lambda}(m) = \frac{m(1-q)^2 - (1-q^2)(1+\rho)}{(1+q)(1+\rho)(1+q+2\rho)}, \tag{64a}$$

$$\lambda_*(m) = \frac{m - (1+\rho)}{\rho(1+\rho) + m(2+\rho)}, \tag{64b}$$

$$\lambda^*(m) = \frac{m - (1+\rho)}{(1+\rho)(1+2\rho)}. \tag{64c}$$

All these three functions are increasing in m. Moreover, λ_* and λ^* are equal to zero at a critical value of m given by

$$\underline{m} = 1 + \rho, \tag{65}$$

while $\bar{\lambda}$ is equal to zero at a critical value of m given by

$$\overline{m} = \frac{(1-q^2)(1+\rho)}{(1-q)^2}, \tag{66}$$

which, since $(1-q^2)/(1-q)^2 > 1$ for $0 < q < 1$, satisfies $\underline{m} < \overline{m}$.

It was already established that $\lambda^* > \lambda_*$. To see how $\bar{\lambda}$ is ordered with respect to λ_* and λ^*, consider first the difference $\bar{\lambda} - \lambda_*$ for $m \geq \overline{m}$. Note that, for $m = \overline{m}$, we have $\bar{\lambda}(\overline{m}) = 0 < \lambda_*(\overline{m})$, and hence $\bar{\lambda} - \lambda_* < 0$. Furthermore, in the limit of large m, we have $\lim_{m\to\infty} \bar{\lambda}(m) = \infty$, and $\lim_{m\to\infty} \lambda_*(m) = 1/(2+\rho)$, so that the difference $\bar{\lambda} - \lambda_*$ is positive when m is large. We then have that, for $m \in [\overline{m}, \infty)$, $\bar{\lambda} - \lambda_*$ has an odd number of sign changes. From (64a) and (64b), we have that $\bar{\lambda} - \lambda_*$ also satisfies

$$\bar{\lambda} - \lambda_* =_s (1-q)^2(2+\rho)m^2 - (1+\rho)\left[3 + 2\rho + q(2-q)(1+2\rho)\right] m + (1+q)^2(1+\rho)^3. \tag{67}$$

Call $p(m)$ the polynomial in m at the right hand side of the above expression. By Descartes' rule of signs, $p(m)$ and hence $\bar{\lambda} - \lambda_*$ has either zero or two sign changes in the interval $[0, \infty)$. Since we have established that $\bar{\lambda} - \lambda_*$ has an odd number of sign changes in $[\overline{m}, \infty)$, it must be that $\bar{\lambda} - \lambda_*$ has two positive roots, one in the interval $[0, \overline{m})$ and another in the interval $[\overline{m}, \infty)$. Moreover, at this latter root, $\bar{\lambda} - \lambda_*$ changes sign from $-$ to $+$.

To find the critical value $\tilde{m}$ at which $\bar{\lambda} = \lambda_*$ we set (67) to zero and solve the resulting quadratic equation to obtain

$$\tilde{m} = \frac{3 + q(2-q)(1+\rho)(1+2\rho) + \rho(5+2\rho)}{2(1-q)^2(2+\rho)} + \frac{(1+\rho)\sqrt{(1+q)^2\left[1+q(10-7q)\right] + 8q(1+q)\left[4-q(1+q)\right]\rho + 16q\left[1+q(1-q)\right]\rho^2}}{2(1-q)^2(2+\rho)}.$$

We then have that $\bar{\lambda} \leq \lambda_*$ for $m \in [\overline{m}, \tilde{m}]$ and $\bar{\lambda} > \lambda_*$ for $m \in [\tilde{m}, \infty)$.

Next, we consider how $\bar{\lambda}$ compares to λ^*. We can show that $\lambda^* > \bar{\lambda}$ holds for all $m \in [\underline{m}, \infty)$. To show this, note that the derivatives of λ^* and $\bar{\lambda}$ with respect to m are given by

$$\frac{d\lambda^*}{dm} = \frac{1}{(1+\rho)(1+2\rho)}$$
$$\frac{d\bar{\lambda}}{dm} = \frac{(1-q)^2}{(1+\rho)(1+q)(1+q+2\rho)}$$

and that they satisfy $d\lambda^*/dm > d\bar{\lambda}/dm$, since we can write

$$\begin{aligned}
\frac{d\lambda^*}{dm} &> \frac{d\bar{\lambda}}{dm} \\
\frac{1}{(1+\rho)(1+2\rho)} &> \frac{(1-q)^2}{(1+\rho)(1+q)(1+q+2\rho)} \\
(1+q)(1+q+2\rho) &> (1-q)^2(1+2\rho) \\
2q + 6q\rho - 2q^2\rho &> 0 \\
2q\left[1+(3-q)\rho\right] &> 0,
\end{aligned}$$

and the last of these conditions always holds for all $0 < q < 1$. Since $d\lambda^*/dm > d\bar{\lambda}/dm$ and $\overline{m}$ (at which $\bar{\lambda} = 0$) is larger than $\underline{m}$ (at which $\lambda^* = 0$), we have that $\lambda^* > \bar{\lambda}$ for all $m \geq \underline{m}$.

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Education

Sep. 2014 – present **PhD in Biology** GEOMAR (Kiel, Germany)
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Thesis: Eco-evolutionary processes in Caribbean reef fish (*Hypoplectrus* spp)
Supervisor: O. Puebla
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2010 – 2013 **MSc in Biology** McGill University (Montréal, Canada) & STRI (Panama)
Thesis: Phylogeography and signal evolution in one species of Neotropical weakly electric fish, *Brachyhypopomus occidentalis*
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2006 – 2009 **BA & Sc in Biology & International Development** McGill University
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Supervisor: R. Krahe

Selected career-related experience

February 2016 & 2017 **Teaching assistant** (Three Seas program, Northeastern University)
Biology and Ecology of Reef Fishes, field course in Bocas del Toro, Panamá

2016 - present **Reviewer** for publications
Journals: *Marine Biology*

January 2015 & 2016 **Teaching assistant** (GEOMAR)
Practical course in DNA-barcoding for Biological Oceanography

January 2014 – June 2014 **Research assistant** (AES Corporation, Panamá)
Environmental impact assessment: monitoring of aquatic species in the hydroelectric power plant of the Changuinola river
ITS Consultores Panamá

October 2013 – June 2014 **Research intern** (STRI Bocas del Toro station, Panamá)
Research project: Pairing dynamics and origin of species in reef fish (*Hypoplectrus* spp)

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June 2013	**Workshop** (Aussois, France) Software and statistical methods for population genetics Dr. M. Blum, CNRS, TIMC-IMAG lab, UJF Grenoble
2011 – 2012	**Teaching assistant** (McGill University) *Methods in Biology of Organisms* *Environmental Management II*
January – May 2010	**Research assistant** (Biodôme de Montréal) Research project: Effect of density on growth and stress level of the Atlantic wolffish (*Anarhichas lupus)* Supervisor: N. Le François

Publications

Picq S, Scotti M, Puebla O. *Under review*. Animal personality, speciation and adaptive radiation: an empirical study in a natural reef fish population. *The American Naturalist*

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Selected oral presentations and posters

Picq S, Puebla O. 2016. Animal personality and speciation in coral reef fishes. Talk at the *16th International Behavioural Ecology Congress*, Exeter, UK.

Hench K, **Picq S**, Theodosiou L, McMillan O, Puebla O. Speciation and local adaptation in coral reef fishes (*Hypoplectrus* spp): a genomic perspective . Talk at the *2016 Annual Symposium of the Fisheries Society of the British Isles*, Bangor, UK.

Picq S, Tran A, Krahe R. 2016. Genetic drift and natural selection as driving forces in the evolution of electric signals in weakly electric fish. Talk at the *12th International Congress of Neuroethology,* Montevideo, Uruguay.

Picq S, McMillan WO, Puebla O. 2015. Genomic architecture of local adaptation versus speciation in coral reef fishes. Poster at the *European Society for Evolutionary Biology*, Lausanne, Switzerland.

Picq S. 2015. Peces de arrecife de Bocas del Toro. Talk of the month at the STRI Bocas del Toro research station, Bocas del Toro, Panama.

Puebla O, **Picq S**, McMillan WO, Bermingham E. March 2015. Mutual mate choice and speciation. Lightening talk and poster at the *Gordon Speciation Conference*, Ventura, USA → Poster award!

Picq S, Puebla O. Mutual mate choice and speciation. March 2015. Talk at the *7th Annual Workshop on Theoretical Biology,* Max Planck Institute for Evolutionary Biology, Plön, Germany.

Nieder C, **Picq S**, Puebla O. March 2014. Long-term pairing dynamics in the hamlets (*Hypoplectrus* spp, Serranidae). Poster at the *Fellows' Symposium*, STRI, Panamá.

Picq S, Alda F, Bermingham E, Krahe R. June 2013. Geographical variation in the electric signals of the Neotropical weakly electric fish *Brachyhypopomus occidentalis*: a strong role for stochasticity in signal evolution? Talk at the *Evolution 2013* conference, Salt Lake City, USA.

Picq S, Alda F, Bermingham E, Krahe R. 2012. Phylogeny and electric signal variation of the primary Neotropical knifefish B*rachyhypopomus occidentalis*. Poster at the *10th International Congress of Neuroethology*, University of Maryland, USA.

Picq S, Bermingham E, Krahe R. 2011. Geographic variation in the electric signals of a Neotropical knifefish: sexual selection, drift, or local adaptation? Invited talk at the *Department of Neurobiology* in Ludwig Maximilians University, Munich, Germany.

Grants and scholarships

2015	STRI Short-term Fellowship	$4,000
2014	IMPRS PhD Scholarship (for three years)	€52,848
2013	Society for the Study of Evolution Award	$500
2013	STRI Fellow Travel Award	$1,000
2011	NEO Grant-in-aid of research	$4,000
2010	STRI-McGill NEO Fellowship	$5,000
2010	McGill International MSc Award	$10,000
2010	Principal's Graduate Award	$2,500
2010	Provost's Graduate Fellowship	$1,500

Students advised

Justin Lesser	M.Sc. student, Three Seas Program (Northeastern University, Boston, USA)	May - Oct. 2015

Research skills

Genetics	DNA extraction, PCR, RAD-sequencing analyses
Computer skills	basic MATLAB® programming, statistical modelling with R, familiar with Unix operating system, ArcGIS
Field Work	boat handling certification, Scuba diving (PADI) advanced diver, +400 logged dives, Sirius® wilderness first aid and safety training
Languages	French, English, Spanish, fluent written and spoken

PAPERS PUBLISHED DURING MY PH.D.

1. Picq S, McMillan OW, Puebla O. 2016. Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp, Serranidae). *Ecology and Evolution* 6 (7): 2109-2124.

2. Picq S, Alda F, Bermingham E, Krahe R. 2016. Drift-driven evolution of electric signals in a Neotropical knifefish. *Evolution* 70 (9): 2134–2144.

This was published during my Ph.D. but was not part of it. It was part of my M.Sc. research:

Abstract
Communication signals are highly diverse traits. This diversity is usually assumed to be shaped by selective forces, whereas the null hypothesis of divergence through drift is often not considered. In Panama, the weakly electric fish *Brachyhypopomus occidentalis* is widely distributed in multiple independent drainage systems, which provide a natural evolutionary laboratory for the study of genetic and signal divergence in separate populations. We quantified geographic variation in the electric signals of 109 fish from five populations, and compared it to the neutral genetic variation estimated fromcytochrome oxidase I (COI) sequences of the same individuals, to test whether drift may be driving divergence of their signals. Signal distances were highly correlated with genetic distances, even after controlling for geographic distances, suggesting that drift alone is sufficient to explain geographic variation in electric signals. Significant differences at smaller geographic scales (within drainages) showed, however, that electric signals may evolve at a faster rate than expected under drift, raising the possibility that additional adaptive forces may be contributing to their evolution. Overall, our data point to stochastic forces as main drivers of signal evolution in this species and extend the role of drift in the evolution of communication systems to fish and electrocommunication.