



Evolution of male antennal size in moths (Lepidoptera): a comparative test of potential trade-offs and correlated growth in Australian species

Sam Weedon, Nicholas Porch and Matthew R E Symonds* 

Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125, Australia.

Abstract

One of the most conspicuous features of many moth species is their antennae, which can be strikingly elaborate. However, the factors that have influenced the evolution of these impressive receptor organs remain poorly known. Antennae are potentially costly structures, and previous research has indicated that investment in these structures may be traded-off against investment in other organs, depending on the mating strategy in which individuals engage. Using a phylogenetic comparative analysis of data from dissected wild-caught individuals from 44 Australian moth species, we examined potential trade-offs and correlations between antennal size (measured as antenna length and antenna area) and the size of a range of other morphological features related to paternity (testis area), and vision (eye diameter). Antenna area did not show any evidence of a trade-off with testis size (area) after controlling for body size and phylogeny. Further, relative antenna length was *positively* correlated with relative eye size, suggesting that investment in both sensory structures is linked. Analysis of the allometric scaling of antennal size and eye diameter found that larger moth species invested relatively more in the size of their male antennae (both area and length) than in the size of their eyes. These results indicate different patterns of investment in sensory structures in relation to body size, with larger moth species favouring the evolution of more elaborate antennae in males.

Key words

allometry, antennal morphology, phylogenetic GLMM, receiver, sexual selection, testis size.

INTRODUCTION

Darwin (1871) proposed in *The Descent of Man* that male animals with relatively larger sensory organs should more easily be able to detect and locate females. Consequently, he argued, sexual selection may act on ‘organs of sense’, such as insect antennae. While a large amount of research has focussed on the evolution of sexually selected traits such as ornaments or weaponry, there has been very little research on this neglected aspect of Darwin’s sexual selection theory (see recent review by Elgar *et al.* 2019).

Perhaps one of the most well-known examples of insects evolving elaborate sensory organs is the antennae of moths (Lepidoptera), which, particularly in males, can be strikingly elaborate and feathery, potentially branching off into as many as four branches in species such as the Luna moth, *Actias luna* (Scoble, 1992). Most moth species, though, have simple, filiform antennae, with branching (pectinate) antennae being comparatively uncommon (Mankin & Mayer 1984; Symonds *et al.* 2012).

Why this variation in antennal morphology exists is still largely unknown. It is presumed that larger antennae (i.e. antennae with greater surface area) may be more sensitive to the signals of female pheromones, which can be broadcast over long distances (Mankin & Mayer 1984; Baker 1989), and there is evidence that males with larger antennae are more likely to locate smaller pheromone sources (such as individual females

compared to multiple females together – Johnson *et al.* 2017a). In scramble competition, where the order of mating influences success (Andersson 1994; Herberstein *et al.* 2017), there should therefore be selection for investment in features that aid in locating and acquiring a mate (pre-copulatory sexual traits; Andersson 1994; Tennessen & Zamudio 2003; Lloyd 1979).

Alternatively, males may invest in post-copulatory sexual traits that prevent a female from mating with other males, including mate plugs or marking pheromones that discourage mating by other males (Drummond 1984; Orr 2002). Additionally, males may invest in traits that provide an individual’s own sperm with an advantage over other males’ sperm. These may involve investing in sperm quantity or length (Drummond 1984; Morrow & Gage 2000), to ensure that male’s sperm fertilise a greater proportion of eggs.

The rarity of elaborate antennae in contrast to the relative ubiquity of long-distance pheromones suggests that there must be costs to growing larger or more elaborate antennae. The costs of a feature may take numerous forms, including resource costs associated with the growth of a feature (Nijhout & Emlen 1998) or energetic costs associated with maintaining the feature (Niven & Laughlin 2008). Elaborate antennae tend to be more common in larger moth species, suggesting that the costs of larger antennae may weigh more heavily on smaller species (Symonds *et al.* 2012). These costs may be realised through trade-offs and compensation with other morphological traits (Reznick 1985; Painting & Holwell 2013).

These trade-offs may manifest themselves as trade-offs between pre-copulatory and post-copulatory traits, the exact degree of which potentially depends on specific environmental

*matthew.symonds@deakin.edu.au

conditions: males of moths that are raised at low population densities tend to develop relatively larger antennae, indicating greater investment in mate detection and location when mates are scarce (pre-copulatory strategies), while those raised at high population densities grow relatively larger testes and have higher sperm counts, indicating investment in traits that ensure a male sires a higher proportion of offspring when sperm competition is likely to be greater (paternity protection – a post-copulatory strategy) (Gage 1995; Johnson *et al.* 2017b). The relationship between testis size and antennal size may not be consistent across species. For example, Shiel *et al.* (2015) found no association between the two traits in the painted apple moth (*Teia anartoides*), a monandrous species. However, most moth species are polyandrous to at least some extent (Drummond 1984; Torres-Vila *et al.* 2004), and it may be that such trade-offs only occur where sperm competition is likely, as this would provide a selective advantage to investing in post-copulatory traits (such as testis size) to ensure that a male produces as many offspring as possible from a mating.

Alternatively, species that are less able to detect pheromones may instead compensate by investing in other senses, and there is some evidence that organisms will trade-off between sexually selected features and sensory systems. For example, Nijhout and Emlen (1998) showed that *Onthophagus taurus* beetles that produced relatively larger horns also produced relatively smaller eyes. For moths, while eyes are still needed to navigate through a landscape, there is considerable evidence that eye size is related to breeding strategy (Javoiš *et al.* 2019). Males use eyes to locate females (Charlton & Cardé 1990), and eye size is typically sexually dimorphic, with males having larger eyes (Rutowksi 2000; Javoiš *et al.* 2019). Given that moth antennae can be characterised as sexually selected traits, it is possible that we may see a such a trade-off between the size of the antennae and the size of the eyes. Contrary to this expectation, Shiel *et al.* (2015) found a positive association between these two sensory traits in *T. anartoides* (Lymantriidae), which they suggested may result either from individuals with larger eyes and antennae having greater capacity to extract resources from the environment or from both sensory structures sharing development from the same imaginal structures.

If the cost of a feature is dependent on body size, the effect of this cost may be detected through allometric relationships (Painting & Holwell 2013), that is, the relationship between the size of a feature and body size of an organism. These allometries can be ontogenetic (within an individual), static (within a population or species) or evolutionary (across species) (Gould 1977; Klingenberg & Zimmermann 1992). From an evolutionary perspective, allometric relationships are said to be hyperallometric or hypoallometric depending on whether a feature is relatively larger in larger or smaller species, respectively. These patterns may relate to differing selection pressures between species with different body sizes. For example, hyperallometry may indicate directional selection for larger traits in larger species or may indicate that there are increased costs associated with bearing a trait at smaller body sizes. Furthermore, differences in allometries between males and females may be indicative of sexual selection, particularly if larger species tend to be more sexually dimorphic

(Dale *et al.* 2007). Therefore, by comparing allometric relationships of sensory and reproductive morphological traits, we can gain insights into whether selection is acting differently on different traits.

Research into the allometry of sexually selected traits and trade-offs has tended to focus on weapons, though it has been met with conflicting results. For example, Durrant *et al.* (2016) found that cockroaches with larger pronotal horns had relatively smaller testes, both within and between species. By contrast, Simmons and Emlen (2006) failed to find any trade-off when comparing horn and testis size across 25 *Onthophagus* beetle species, though they did find that there were differing patterns of growth within species. Species with a steeper allometric slope in testis size had a reduced allometric slope in their horn size.

By contrast with this research on ornaments and weaponry, there has been much less focus on sensory traits (Elgar *et al.* 2019), and very few have looked at the associations in a comparative cross-species context. One exception is the recent study of firefly species by Stanger-Hall *et al.* (2018), which found that nocturnal species tend to have shorter antennae but larger eye size, presumably because such species tend to use photic signals to attract and locate mates. Here, we investigate correlates of the size and structure of antennae across a range of Australian moth species, specifically looking at possible trade-offs and compensation in relation to relative testis size and eye size, as well as the allometry of these features across species. We predicted that if male moths face a potential trade-off between their ability to locate a mate and ensuring that they father a greater number of offspring, this would present itself as a negative relationship between antenna size and testis size, after controlling for body size. Note that we acknowledge that a negative correlation is not conclusive proof of a causal trade-off, but that simply state that we would expect to see a negative relationship between the two traits *if* there were a trade-off.

Because the costs associated with bearing elaborate antennae must weigh more heavily on smaller moths (Symonds *et al.* 2012), we predicted that there should be hyperallometry in antennae size. Additionally, we hypothesise that smaller moths may compensate for a decreased olfactory sense by increasing investment in visual systems. This may be particularly so given that reduction in eye size disproportionately affects visual acuity, so smaller moths may not be as able to ‘sacrifice’ their eye size as their larger relatives (Land 1997). We therefore predicted hypoallometry for eye size (larger individuals have relatively smaller eyes). A hypoallometric relationship between eye size and body size has been found across other lepidopteran species (Rutowksi 2000). As a consequence of the differing predicted allometries of antennal size and eye size, we also predicted a negative relationship between the size of the eyes and the size of the antennae (after controlling for body size).

Sexual dimorphism in the antennae and eyes of Lepidoptera is very common (Scoble 1992; Rutowksi 2000; Javoiš *et al.* 2019). If males and females show similar patterns in the associations between sensory traits or in the allometries of those traits, this may suggest that the patterns are the result of an environmental selective pressure, rather than a sexually selective pressure. We therefore also examined associations and allometry in

sensory traits of females as well as males, in order to better establish if any trade-offs or allometric patterns are the result of natural, rather than sexual, selection. Since males should be under more intense sexual selection for these sensory traits, we predicted that they may be more likely to show trade-offs with antenna size and steeper allometries in sensory traits than females.

MATERIALS AND METHODS

Collection

We collected moth specimens by light trapping between September 2016 and February 2017 from field sites

predominantly in Victoria, south-eastern Australia. Specifically, the trapping localities were the eastern suburbs of Melbourne (Ringwood (37°47'47"S, 145°13'6"E) and Lilydale (37°46'15"S, 145°21'50"E), Mt. Hotham Alpine Resort (36°59'41"S, 147°9'8"E), Skipton (37°41'10"S, 143°22'9"E) and Bradvale (37°47'57"S, 143°25'2"E), and Kambah, Australian Capital Territory (35°22'39"S, 149°3'52"E)) (Fig. 1). Specimens were attracted using an ultraviolet light (BioQuip 15 W, 12 V black light), powered by a 12 V battery and illuminated with white light for ease of collection. The lights were hung in front of a white sheet suspended approximately 2 m above the ground. The trap was set up around sunset and left for approximately an hour before specimen collection.

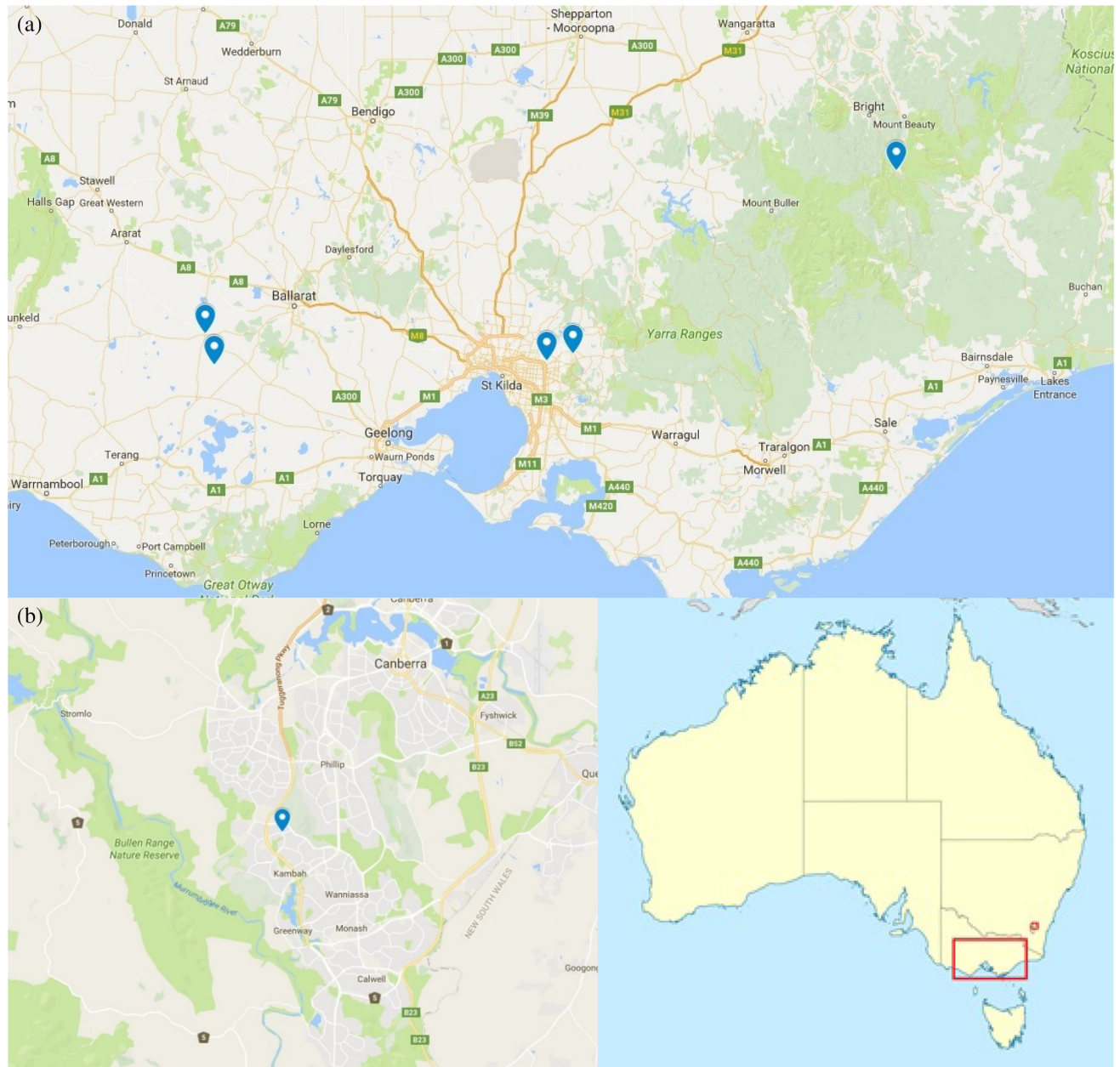


Fig. 1. Collection localities (blue markers) in (a) Victoria and (b) Australian Capital Territory. (c) Areas of Australia covered by (a) and (b). Maps of (a) and (b) made using Google MyMaps (Google 2017); (c) derived from Tann (2017).

Specimens that were to be collected were photographed for later identification and then killed with ethyl acetate before being placed in 70% ethanol for preservation. Which taxa were sampled was primarily determined by the species that were actually trapped at each collection locality and were not otherwise selected based on diversity or abundance.

Sexing and identification

Where possible, specimens were identified to species level by visual comparison of the wing colouration patterns, though in some cases, they could only be identified to genus. Specimens were identified using the *Moths of Australia* book (Common 1990) and reference to the Atlas of Living Australia (www.ala.org.au) and Bowerbird (www.bowerbird.org.au) Australian biodiversity websites. A total of 212 individuals were identified representing 44 species from 11 moth families (Table 1). Specimens were sexed by examining the frenulum on the underside of the wing, which is sexually dimorphic, with males having a single, thick bristle, while females have two or more thinner, hair-like bristles (Common 1990).

Dissection

We dissected specimens under a Nikon SMZ 745 microscope using a small scalpel and forceps. A single antenna and a foreleg were removed from each specimen. The right-hand appendage was preferred (from the viewer's perspective – mounted in the ventral view); however, in case of breakage, the left-hand appendage was substituted. Some dissections resulted in both appendages breaking, in which case the appendage of that specimen had to be excluded from analysis. The head was also removed for eye measurement.

For male specimens, the testis was removed by dissecting the cuticle between the third and fourth segments forward of the genitalia and gently removing the white tissue mass (duplex) in order to expose the testis (Shiel *et al.* 2015). The testis could be distinguished from the duplex by either its colour (typically coloured purple or white; Emmel 1968; Gage 1995) or its defined, spherical shape, in contrast to the shapeless, white, soft tissue of the duplex.

Photographing and measurement of body parts

Auto-montaged photographs of each body part (antenna, foreleg, head and testis) were taken using a Leica M205C stereomicroscope with a 1.0× plan apo objective. A scale bar was embedded into the resulting image for subsequent measurement and analysis in the free software ImageJ (Schneider *et al.* 2012).

Foreleg tibia length was used as a measure of overall body size for this study (Teuscher *et al.* 2009). The length of the tibia was measured as the longest straight-line distance along the tibia from near the joint with the tarsus to the joint with the femur (Fig. 2a).

Images of the antennae were taken so as to have all branches visible in the image if they were present. Antenna size is not necessarily as straightforward as a simple length of the main antenna (flagellomere), as branches can increase

the area dramatically without increasing the length (Symonds *et al.* 2012). Therefore, three measurements from each antenna were made so as to account for this variation: the length, along the centre line of the flagellomere; the area, by taking the polygonal area of the antenna around the tips of the branches or hairs (Fig. 2b; Shiel *et al.* 2015); and the count of the number of lateral branches on the antenna. Pectinate antennae are rare in females (Scoble 1992). As such, although some female specimens did possess pectinate antennae, only the length of the female antennae was measured.

In some cases of species with bipectinate antennae, it was not possible to position the antenna so that all branches were evenly visible due to the small angle formed by the branches. In these cases, two images were taken, each clearly displaying one side of the antenna. For measurement of these antennae, a measurement of the area of one side of the branches was made, including the flagellomere, and a subsequent measurement was made of the branches on the other side, excluding the flagellomere. The sum of these two measurements was then taken to be the measurement of the antennal area. Areas were not measured for filiform antennae.

In keeping with previous work (Shiel *et al.* 2015; Rogers *et al.* 2005), testis area was used as a measure of testis size, measuring the largest polygonal area of the testis (Fig. 2c). Likewise, images of the head were always taken in the 'front-on' (or proximal) view, with the antennae (or position thereof) towards the top of the image and the proboscis in the centre. The vertical diameter of the eye at its widest was taken as the measurement of eye size (Shiel *et al.* 2015; Fig. 2d).

Statistical analysis

Species that are closely related phylogenetically are likely to be more similar due to relatedness than more distantly related species, meaning that they are not statistically independent of each other (Harvey & Pagel 1991; Grafen 1989). Therefore, we controlled for phylogeny by using species as a random factor in a phylogenetic generalised linear mixed model. There was no comprehensive phylogeny for all species collected, and so, we constructed a composite phylogeny (see Beaulieu *et al.* 2012, for justification of this approach). The phylogeny was constructed using a 'backbone' phylogeny that establishes the species relationships between the larger groups (Regier *et al.* 2013) and then resolving the relationships within those groups using other phylogenies (Young 2006; Mitchell *et al.* 2006; Zahiri *et al.* 2011; Sihvonen *et al.* 2011). As part of the construction of the phylogeny, where species-level resolutions were not possible, some taxonomic information was used to resolve relationships; thus, species in the same genus were assumed to be sibling species. In the absence of accurate branch length information, we used Grafen's (1989) algorithm to estimate branch length, where the depth of a node in the tree is equal to the number of daughter species descended from that node. The phylogeny was put together using the software Mesquite v3.2 (Maddison & Maddison 2017) and is shown in Figure 3.

Table 1 Moth species collected for the study, with species mean measurements (all in mm or mm²) and sample sizes for males and females

Family	Species	Tibia length (male)	Tibia length (female)	Antenna length (female)	Antenna length (male)	Antenna area	Antenna no. of branches	Eye diameter (male)	Eye diameter (female)	Testis area	Male (n)	Female (n)
Anthelidae	<i>Anthela acuta</i>	4.22	—	10.48	—	22.41	107	1.45	—	0.61	4	0
Arctiidae	<i>Anestia ombrophanes</i>	1.71	—	4.45	—	1.75	54	0.64	—	—	2	0
	<i>Spilosoma curvata</i>	3.42	—	8.47	—	8.38	80	1.20	—	0.74	1	0
	<i>Spilosoma glatignyi</i>	4.08	—	10.01	—	—	99	1.39	—	3.20	4	0
Crambidae	<i>Achyra affinitalis</i>	2.60	2.34	7.78	6.48	—	—	0.83	0.67	0.74	2	1
	<i>Nechilo macrogona</i>	2.77	2.79	8.45	8.64	—	—	0.94	0.91	0.28	1	0
	<i>Scoparia exhibitalis</i>	1.83	1.99	4.95	5.57	—	—	0.72	1.05	0.22	2	2
Depressariidae	<i>Enchocrates glaucopis</i>	1.59	6.90	—	—	—	—	0.64	—	1.59	3	0
Geometridae	<i>Chloroclystis filata</i>	—	2.28	—	5.69	—	—	—	0.77	—	0	6
	<i>Chloroclystis testulata</i>	—	1.93	—	5.25	—	—	—	0.72	—	0	14
	<i>Chlorocoma dichloraria</i>	2.78	2.67	6.96	6.95	5.47	62	0.93	0.76	0.12	2	1
	<i>Chrysolarentia plagiocasta</i>	—	2.87	—	7.35	—	—	—	0.91	—	0	1
	<i>Cyneoterpna sp.</i>	4.60	—	13.19	—	15.44	138	1.54	—	4.60	3	0
	<i>Ectropis fractaria</i>	3.63	—	9.17	—	3.73	—	—	—	—	1	0
	<i>Epicyme rubropunctaria</i>	—	2.16	—	5.32	—	—	—	0.64	—	0	1
	<i>Epidesmia chilonaria</i>	4.06	—	10.88	—	4.37	56	1.23	—	4.06	1	0
	<i>Epidesmia tryxaria</i>	1.61	—	9.25	—	3.67	48	1.10	—	0.47	2	0
	<i>Epyaxa subidaria</i>	—	2.73	—	7.13	—	—	—	0.86	—	0	1
	<i>Euphronarcha luxaria</i>	3.49	3.57	9.12	9.45	7.35	92	1.10	1.03	—	1	1
	<i>Melanodes anthracitaria</i>	4.59	—	12.26	—	3.03	64	1.37	—	0.77	2	0
	<i>Phelotis cognata</i>	2.58	2.48	6.70	6.69	7.39	60	0.82	0.72	0.51	6	14
	<i>Phrissogonus laticostata</i>	2.00	1.92	5.18	5.07	—	—	0.75	0.69	—	4	16
	<i>Sandava scitisignata</i>	2.30	—	7.04	—	4.22	52	0.92	—	0.50	1	0
	<i>Scopula optivata</i>	2.71	—	6.23	—	—	—	0.68	—	—	1	0
	<i>Scopula rubraria</i>	2.62	2.40	6.52	6.51	3.96	—	0.72	0.65	0.21	3	2
	<i>Syneora hemeropa</i>	3.15	—	9.11	—	6.21	64	1.22	—	—	1	0
Lasiocampidae	<i>Entometa fervens</i>	—	4.81	—	9.09	—	—	—	1.53	—	0	1
Noctuidae	<i>Agrotis infusa</i>	4.39	4.67	13.82	14.41	6.29	105	1.64	1.64	1.59	24	27
	<i>Agrotis munda</i>	3.60	3.65	11.59	11.49	4.69	93	1.51	1.40	0.94	2	8
	<i>Arrade leucocosmalis</i>	—	2.24	—	7.03	—	—	—	0.79	—	0	1
	<i>Chrysodeixis argentifera</i>	3.66	3.46	10.11	10.96	—	—	1.39	1.41	1.46	1	1
	<i>Helicoverpa punctigera</i>	—	3.55	—	9.68	—	—	—	1.33	—	0	4

(Continues)

Table 1 (Continued)

Family	Species	Tibia length (male)	Tibia length (female)	Antenna length (female)	Antenna length (male)	Antenna area	Antenna no. of branches	Eye diameter (male)	Eye diameter (female)	Testis area	Male (n)	Female (n)
	<i>Helicoverpa</i> sp.	3.79	3.67	10.97	11.88	—	—	1.57	1.46	2.16	1	1
	<i>Hypoperigea tonsa</i>	—	2.67	—	8.38	—	—	1.14	—	—	0	1
Nolidae	<i>Uraba lugens</i>	5.89	—	5.89	—	2.55	73	0.42	—	0.24	1	0
Oecophoridae	<i>Euchaetis rhizobola</i>	0.99	2.56	—	9.87	—	—	—	1.01	—	0	1
	<i>Philobota latifissella</i>	1.75	1.77	7.66	7.72	—	—	0.65	0.66	0.14	6	2
	<i>Philobota orescoa</i>	1.40	—	6.37	—	—	—	0.65	—	0.13	8	0
	<i>Philobota partitella</i>	1.65	—	6.63	—	—	—	0.72	—	0.26	2	0
	<i>Tachystola acroxantha</i>	0.99	—	3.52	—	—	—	0.42	—	—	1	0
Pyralidae	<i>Hellula hydralis</i>	—	1.90	—	5.41	—	—	—	0.67	—	0	3
	<i>Mimaglossa</i> sp.1	2.45	—	6.44	—	—	—	0.81	—	0.31	1	0
	<i>Nomophila corticalis</i>	—	—	—	—	—	—	—	—	—	2	1
Tortricidae	<i>Epiphyas postvittana</i>	1.61	—	4.71	—	—	—	0.68	—	0.23	2	0

We carried out phylogenetic generalised mixed model analyses using the *MCMCglmm* package in R (Hadfield 2010), which accounts for intraspecific variation as well as interspecific variation using a Bayesian Markov chain Monte Carlo approach. In analyses, we assumed default ‘uninformative’ priors ($\nu = 1$, $\nu_0 = 0.02$) (De Villemereuil & Nakagawa 2014). Morphological variables were included as fixed factors, with testis size, eye diameter and tibia length being included as predictor variables and antenna size (number of branches, area and length) as Gaussian response variables and species identity as a random factor with the phylogenetic relatedness included in the model variance structure (Hadfield & Nakagawa 2010). Analyses were conducted using 1 001 000 iterations, with a burn-in of 1000 and a thinning factor of 500, resulting in an effective sample size of 2000 estimates that comprised the posterior distribution. Analyses were conducted separately for males and females. Visual examination of Bayesian chain plots was used to confirm convergence of estimates.

We conducted allometric analyses, where we compared tibia length (predictor) with other morphological features (response) while including species as a random factor. All measurements were log transformed prior to analysis, in order to be able to interpret the exponent of the allometric relationships (Huxley 1932; Mascaro *et al.* 2014). We sought to evaluate whether the estimated allometric relationships differed from isometry. Note that, under isometry, linear measurements (antenna length, eye diameter and number of branches) exhibit an allometric slope equal to 1, whereas areal measurements (antenna area and testis area) exhibit an allometric slope equal to 2.

For analysis of the relationships between morphological features and antennal length, area or number of branches (response),

we analysed each predictor variable (testis area and eye diameter) in separate models (due to their high collinearity). For tests of antenna size, antenna area could not be compared with testis size due to the small number of species for which we had both antennal area and testis size measurements. Since larger individuals will have absolutely larger organs, we also included foreleg (tibia) length in these models, in order to control for body size. Note that this approach is recommended rather than using residual values (Freckleton 2002). However, for visualisation of the relationship between relative trait sizes, we did use the residuals of the relationships of those traits against tibia length (i.e. from the allometric analyses above).

RESULTS

Evolutionary allometry of sensory structures

All features showed significant (all pMCMC values < 0.001) positive relationships with body size (tibia length) across species (Table 2); however, there was evidence of some sex differences in allometry. In males, antennae showed isometry (slopes not different from 1, for linear measures, or 2 for areal measures) when measured by their length (Fig. 4a), area and number of branches, although area and number of branches showed a tendency towards hypoallometry (95% credibility intervals from the Bayesian posterior distributions only just include 2 and 1, respectively). Eye diameter in males showed clear hypoallometry (slope is significantly less than 1, Fig. 4b), while testis area showed an isometric scaling relationship with body size. In females, by contrast with males, antennae length showed hypoallometry, while eye diameter was statistically

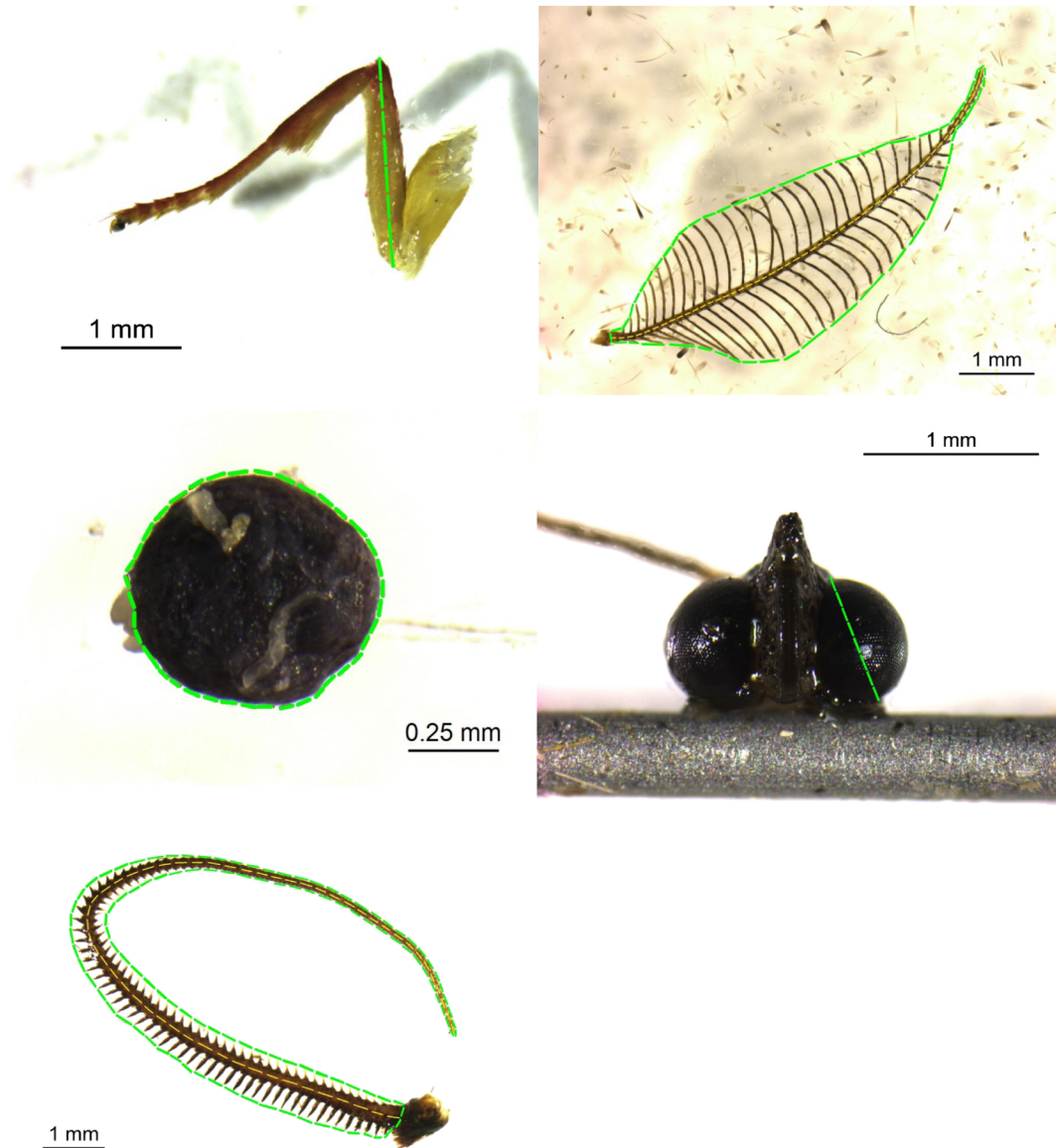


Fig. 2. Measurements of morphological features from a range of Lepidoptera species: (a) tibia, measuring length, (b) antenna, measuring area and length, (c) testis, measuring area, (d) eye, measuring diameter, and (e) antenna of *Agrotis infusa*, measuring area and length, showing the diversity of forms in elaborate antennae compared with (b). Images are not all from the same specimen.

indistinguishable from isometry although it tended towards hypoallometry (Table 2).

Relationships between antennal measures and other morphology

When comparing the relationships between features (Table 3), after controlling for body size, only eye diameter showed a strong positive relationship with antenna length in males (Fig. 5) but not in females. In males, antennal area and number of branches did not show significant relationships with eye diameter, although the latter showed a tendency towards a positive relationship (Table 3). Finally, testis size did not show a significant relationship with antenna length (Fig. 6).

DISCUSSION

No trade-off between antenna size and testis size

Pre-copulatory sexual traits can be expensive, and if an organism is to invest in them, they may face trade-offs with post-copulatory sexual traits (Nijhout & Emlen 1998). Therefore, in the case of moths, we may expect to see a negative relationship between the size of their antennae (a pre-copulatory sexual trait) and the size of their testes (indicating investment in post-copulatory sexual traits). Contrary to these expectations, however, testis size did not show a significant relationship with antenna length in the moth species in this analysis.

This lack of pattern may have two possible explanations. First, males may have other means of ensuring success in post-copulatory competition other than simply sperm quantity – such

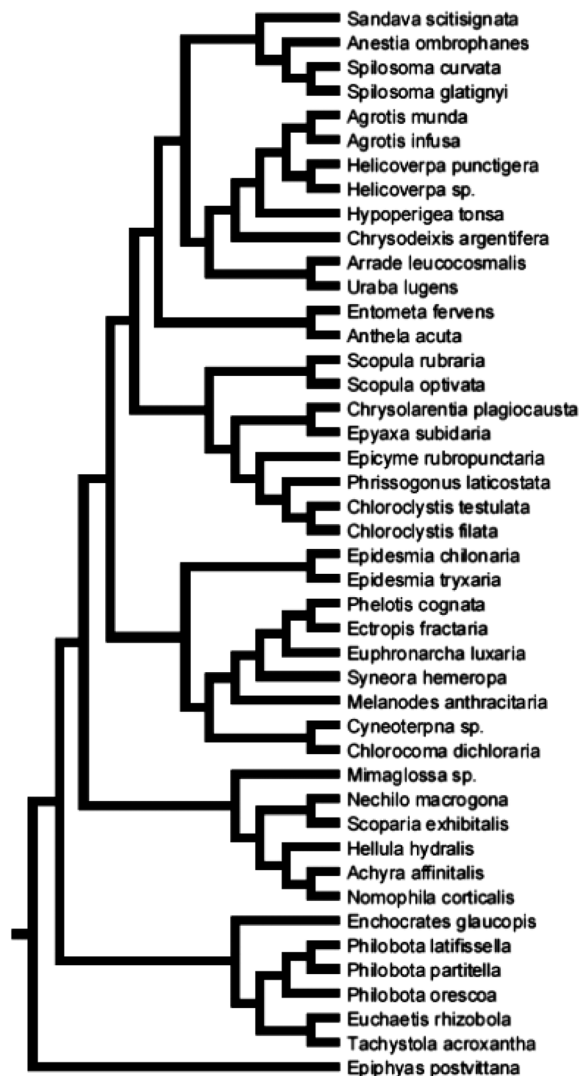


Fig. 3. Phylogeny of 44 collected moth species from south-eastern Australia. Phylogeny constructed using prior literature in Mesquite 3.2 (Maddison & Maddison 2017).

as mate plugs, prolonged copulation and marking pheromones that discourage potential rivals (Drummond 1984). Second, species in temperate regions are short-lived and tend to mate once and then die before they can subsequently remate (Drummond 1984). We do not have data on the mating systems for most of the species that we collected; however, given the temperate climate of Victoria, Australia, many of them may have effectively monandrous females. Therefore, males may not need to invest in testis size as a paternity protection measure, as the females do not remate, thus incentivising investment in sensory structures to mate with more females (Shiel *et al.* 2015). If we were to examine species from a wider variety of climates, and more polyandrous mating systems, it is possible that we are more likely to observe a differing investment strategy that prioritises investment in testis or, alternatively, a potential trade-off between testis size and antenna size in species where males engage in multiple mating strategies.

Table 2 Interspecific allometric relationships of morphological traits in 44 species of Australian Lepidoptera, using tibia length as a measure of body size

	Sex	Estimated allometric slope	Lower - 95%	Upper - 95%	pMCMC	<i>n</i>	Allometry
Antenna length	M	0.958	0.849	1.07	<0.001	81	Isometry
Eye diameter	F	0.794	0.636	0.960	<0.001	95	Hypoallometry
Testis area	M	0.749	0.617	0.883	<0.001	88	Hypoallometry
Antenna area	F	0.835	0.643	1.040	<0.001	91	Isometry
No. of branches	M	2.070	1.19	2.89	<0.001	58	Isometry
Antenna area	M	1.330	0.657	2.02	<0.001	45	Isometry
No. of branches	M	0.737	0.446	1.01	<0.001	46	Isometry

All measures were log transformed, and analyses were controlled for phylogeny. Estimated allometric slope is the mean slope estimate of the Bayesian posterior distribution, with the 95% credibility intervals of the mean. pMCMC value indicates the proportion of values in the posterior distribution that are less than 0 and can be interpreted in a similar way to a standard *P*-value. Allometry column states whether the allometric slope estimate indicates hypoallometry or isometry.

Antenna size and eye size

The relationships between antenna size and eye size were also contrary to the idea that moths trade-off investment in antenna size and eye size. Indeed, in males (but not females), there was a *positive* relationship between antenna length and eye diameter independent of body size. This is similar to what has been observed intraspecifically with the painted apple moth, *Teia anartoides* (Shiel *et al.* 2015). Likewise, the number of antennal branches tended towards a positive (but non-significant) relationship with eye diameter.

The evolutionary allometric relationships of antenna size and eye diameter did, however, provide some indirect evidence for differential investment in sensory structures across species. For males, antenna length showed a steeper scaling relationship with body size (slope and 95% CI = 0.958, 0.849–1.070), than eye diameter (0.749, 0.617–0.883). This suggests that as the average body size of males in a species increases, there is a tendency to invest relatively more in antenna length than in eye diameter.

Larger moth species tend to exist at lower population densities (Currie 1993), and elaborate antennae tend to be more common in larger species (Symonds *et al.* 2012; Javoš *et al.* 2019). This could explain the observed allometries of antenna length and eye diameter. Visual signals should only be effective at shorter distances, compared to the long-distance signalling of pheromones (Scoble 1992), as a potential mate must be within line of sight, while this is not necessary for pheromones. Given this, males of larger species are expected to invest relatively more in the size of their antennae, as the female-produced sex pheromones must be detected over long distances, particularly so in species living at lower population densities. This idea is supported by the result that females exhibit a shallower (and hypometric) allometric slope for antenna length (0.794, 0.636–

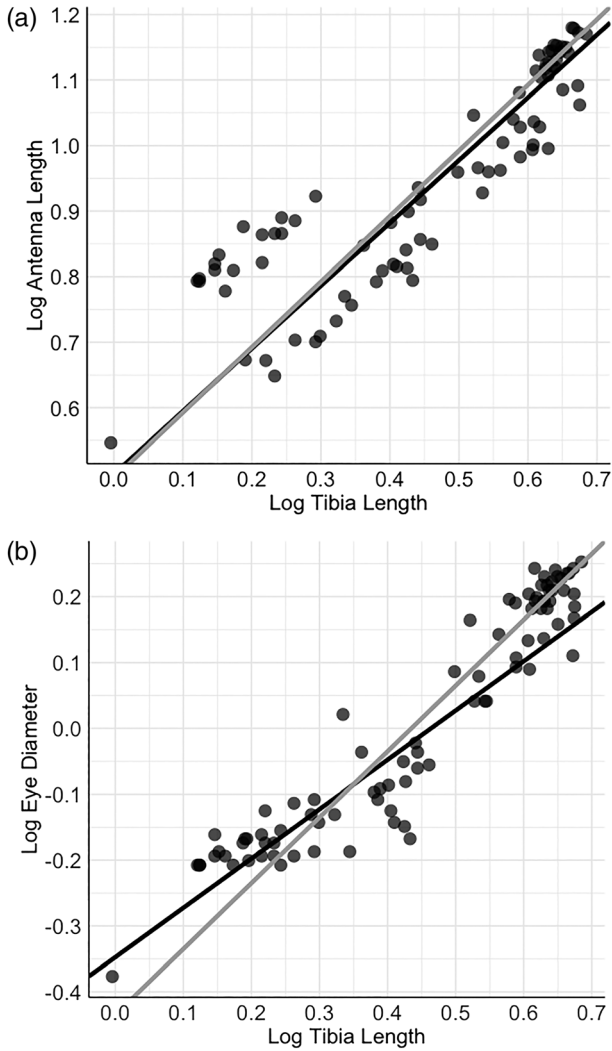


Fig. 4. Regression plots for the interspecies allometry of (a) antenna length and (b) eye diameter in males, with tibia length as a measure of overall body size, controlled for phylogeny. All measures are in mm and log transformed. Calculated allometric slopes from MCMCglmm are shown (black line) along with the predicted relationship under isometry (grey line).

0.960), suggesting that larger species exhibit greater dimorphism in antenna length. Additionally, for males, lower population densities in larger species may result in comparatively weaker selection for larger eye size, since those males rely primarily on olfactory cues to find females.

Table 3 Morphological relationships of interspecies sample of Lepidoptera, controlling for phylogeny and body size (tibia length)

Antenna measure	Sex	Estimate	Lower – 95%	Upper – 95%	pMCMC	<i>n</i>	
Length	Eye diameter	M	2.430	1.25	3.75	<0.001	80
		F	1.273	-0.411	2.86	0.130	86
Area	Testis area	M	0.120	-0.154	0.412	0.400	53
	Eye diameter	M	-0.485	-6.01	5.66	0.854	44
No. of branches	Eye diameter	M	23.1	-3.54	48.5	0.070	46

Estimate indicates the mean slope estimate of the Bayesian posterior distribution, with the 95% credibility intervals of the mean. pMCMC value indicates the proportion of values in the posterior distribution that are less than 0 and can be interpreted in a similar way to a standard *P*-value.

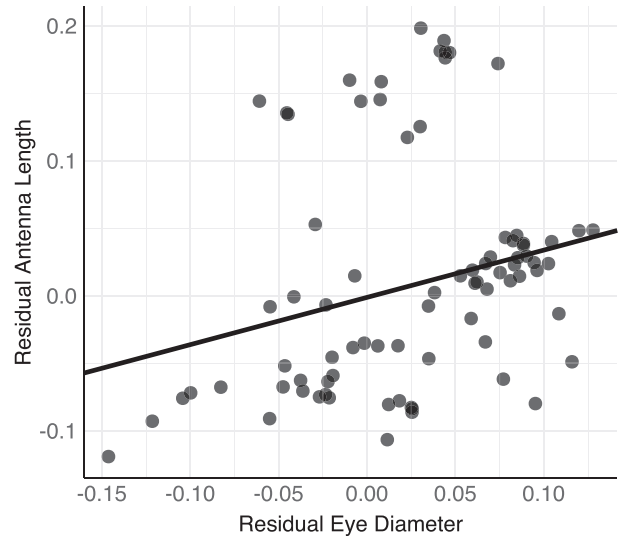


Fig. 5. Partial regression plot of eye diameter and antenna length residuals for interspecies analysis in males after controlling for body size and phylogeny. Measures are residuals from the regression line of the trait against tibia length (log transformed). MCMCglmm calculated regression line is also shown.

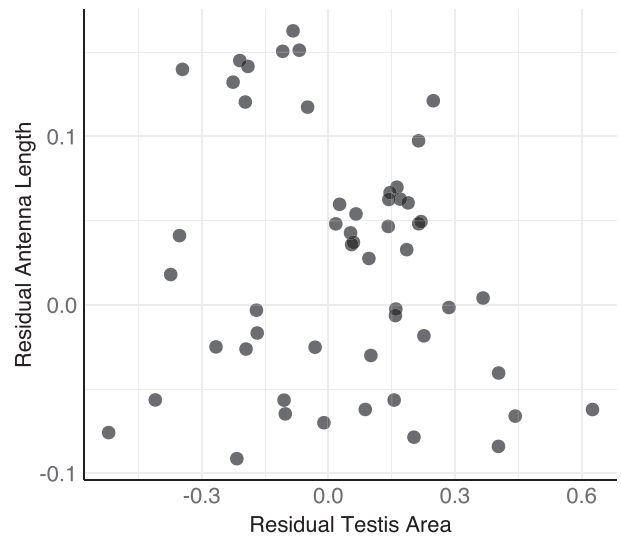


Fig. 6. Partial regression plot comparing residuals of testis area and antenna length in males across Lepidoptera species. Analysis controlled for body size and phylogeny. Measures are residuals from the regression line of the trait against tibia length (log transformed).

The relationship of eye and antenna size to body size in males contrasts with the relationship amongst females. Here, antenna length shows a shallower allometric slope than eye diameter, which shows isometry (0.835, 0.643–1.04). This could be explained by the relative importance of each sensory system to each sex. Males tend to release short-range pheromones, with long-distance pheromones being quite rare (Hansson 1995; Svensson 1996). Given this, we might expect that females would not tend to invest as much in the size of their antennae, as larger antennae are more useful in long-distance communication. Conversely, females may invest relatively more in the size of their eyes, as visual signals provide important information in mate choice and host plant selection.

ACKNOWLEDGEMENTS

We thank Dr Simon Wardrop and Dr Martin Wardrop for their comments and suggestions throughout the duration of this project. We also thank Megan Taylor, Melissa Griffin and Nicholas Reynolds for their assistance in the collection of samples, as well as the community at the online forum, BowerBird, for their great assistance in sample identification. All funds for the research were provided by the School of Life and Environmental Sciences, Deakin University.

REFERENCES

- Andersson MB. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Baker TC. 1989. Sex pheromone communication in the Lepidoptera: new research progress. *Cellular and Molecular Life Sciences* **45**, 248–262.
- Beaulieu JM, Ree RH, Cavender-Bares J, Weiblen GD & Donoghue MJ. 2012. Synthesising phylogenetic knowledge for ecological research. *Ecology* **93**, S4–S13.
- Charlton RE & Cardé RT. 1990. Orientation of male gypsy moths, *Lymantria dispar* (L.), to pheromone sources: the role of olfactory and visual cues. *Journal of Insect Behavior* **3**, 443–469.
- Common IB. 1990. *Moths of Australia*, Vol. 1990. Melbourne University Press, Carlton, Vic.
- Currie DJ. 1993. What shape is the relationship between body size and population density? *Oikos* **66**, 353–358.
- Dale J, Dunn P.O, Figuerola J, Lislevand T, Székely T. and Whittingham L. A. 2007. Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proceedings of the Royal Society of London B*, 274, 2971–2979
- Darwin C. 1871. *The Descent of Man*. Prometheus Books, Amherst, NY 1998.
- De Villemereuil P & Nakagawa S. 2014. General quantitative genetic methods for comparative biology. In: *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology* (ed LZ Garamszegi), pp. 287–303. Springer-Verlag, Berlin.
- Drummond BA. 1984. Multiple mating and sperm competition in the Lepidoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (ed RL Smith). Academic Press, Orlando.
- Durrant KL, Skicko IM, Sturrock C & Mowles SL. 2016. Comparative morphological trade-offs between pre- and post-copulatory sexual selection in Giant hissing cockroaches (Tribe: Gromphadorhini). *Scientific Reports* **6**, 36755.
- Elgar MA, Johnson TL & Symonds MRE. 2019. Sexual selection and organs of sense: Darwin's neglected insight. *Animal Biology* **69**, 63–82.
- Emmel TC. 1968. Methods for studying the chromosomes of Lepidoptera. *Journal of Research on the Lepidoptera* **7**, 23–28.
- Freckleton RP. 2002. On the misuse of residuals in ecology: regression of residuals vs. multiple regression. *Journal of Animal Ecology* **71**, 542–545.
- Gage M.J. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proceedings of the Royal Society of London B: Biological Sciences*, 261, 25–30
- Gould SJ. 1977. *Ontogeny and Phylogeny*. Harvard University Press, Cambridge, Mass.
- Grafen A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **326**, 119–157.
- Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33**, 1–22.
- Hadfield JD & Nakagawa S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. *Journal of Evolutionary Biology* **23**, 494–508.
- Hansson BS. 1995. Olfaction in Lepidoptera. *Experientia* **51**, 1003–1027.
- Harvey PH & Pagel MD. 1991. *The Comparative Method in Evolutionary Biology*, Vol. 239. Oxford University Press, Oxford.
- Herberstein ME, Painting CJ & Holwell GI. 2017. Scramble competition polygyny in terrestrial arthropods. *Advances in the Study of Behavior* **49**, 237–295.
- Huxley JS. 1932. *Problems of Relative Growth*. Dial Press, New York.
- Javoš J, Davis RB & Tammaru T. 2019. A comparative morphometric study of sensory capacity in geometrid moths. *Journal of Evolutionary Biology* **32**, 380–389.
- Johnson TL, Symonds MRE & Elgar MA. 2017a. Sexual selection on receptor organ traits: younger females attract males with longer antennae. *The Science of Nature* **104**, 44.
- Johnson TL, Symonds MRE & Elgar MA. 2017b. Anticipatory flexibility: larval population density in moths determines males investment in antennae, wings and testes. *Proceedings of the Royal Society of London B: Biological Sciences* **284**, 20172087.
- Klingenberg CP & Zimmermann M. 1992. Static, ontogenetic and evolutionary allometry: a multivariate comparison in nine species of water striders. *American Naturalist* **140**, 601–620.
- Land MF. 1997. Visual acuity in insects. *Annual Reviews of Entomology* **42**, 147–177.
- Lloyd JE. 1979. Sexual selection in luminescent beetles. In: *Sexual Selection and Reproductive Competition in Insects* (eds MS Blum & NA Blum), pp. 293–342. Academic Press, New York.
- Maddison, W. P. and Maddison D.R., 2017, Mesquite: a modular system for evolutionary analysis, Version 3.2, Available from URL: <http://mesquiteproject.org>. Accessed 15th March 2017.
- Mankin RW & Mayer MS. 1984. The insect antenna is not a molecular sieve. *Experientia* **40**, 1251–1252.
- Mascaro J, Litton CM, Hughes RF, Uowolo A & Schnitzer SA. 2014. Is logarithmic transformation necessary in allometry? Ten, one-hundred, one-thousand times yes. *Biological Journal of the Linnean Society* **111**, 230–233.
- Mitchell A, Mitter C & Regier JC. 2006. Systematics and evolution of the cutworm moths (Lepidoptera: Noctuidae): evidence from two protein coding nuclear genes. *Systematic Entomology* **31**, 21–46.
- Morrow EH & Gage MJ. 2000. The evolution of sperm length in moths. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, 307–313.
- Nijhout HF & Emlen DJ. 1998. Competition among body parts in the development and evolution of insect morphology. *Proceedings of the National Academy of Sciences* **95**, 3685–3689.
- Niven JE & Laughlin SB. 2008. Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology* **211**, 1792–1804.
- Orr AG. 2002. The sphragis of *Heteronympha penelope* Waterhouse (Lepidoptera: Satyridae): its structure, formation and role in sperm guarding. *Journal of Natural History* **36**, 185–196.
- Painting CJ & Holwell GI. 2013. Exaggerated trait allometry, compensation and trade-offs in the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*). *PLoS One* **8**, e82467.
- Regier JC, Mitter C, Zwick A et al. 2013. A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). *PLoS One* **8**, e58568.
- Reznick D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**, 257–267.

- Rogers DW, Chapman T, Fowler K & Pomiankowski A. 2005. Mating-induced reduction in accessory reproductive organ size in the stalk-eyed fly *Cyrtodiopsis dalmanni*. *BMC Evolutionary Biology* **5**, 37.
- Rutowski R. 2000. Variation of eye size in butterflies: inter- and intraspecific patterns. *Journal of Zoology* **252**, 187–195.
- Schneider CA, Rasband WS & Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671.
- Scoble MJ. 1992. *The Lepidoptera: form, function, and diversity*. Oxford University Press, Oxford; New York 1992.
- Shiel BP, Sherman CD, Elgar MA, Johnson TL & Symonds MRE. 2015. Investment in sensory structures, testis size, and wing coloration in males of a diurnal moth species: trade-offs or correlated growth? *Ecology and Evolution* **5**, 1601–1608.
- Sihvonen P, Mutanen M, Kaila L, Brehm G, Hausmann A & Staude HS. 2011. Comprehensive molecular sampling yields a robust phylogeny for geometrid moths (Lepidoptera: Geometridae). *PloS One* **6**, e20356.
- Simmons LW & Emlen DJ. 2006. Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences*. **103**, 16346–16351.
- Stanger-Hall KF, Sander Lower SE, Lindberg L, Hopkins A, Pallansch J & Hall DW. 2018. The evolution of sexual signal modes and associated sensor morphology in fireflies (Lampyridae, Coleoptera). *Proceedings of the Royal Society of London B* **285**, 20172384.
- Svensson M. 1996. Sexual selection in moths: the role of chemical communication. *Biological Reviews* **71**, 113–135.
- Symonds MRE, Johnson TL & Elgar MA. 2012. Pheromone production, male abundance, body size, and the evolution of elaborate antennae in moths. *Ecology and Evolution* **2**, 227–246.
- Tann J. 2017. Australia medium-res locator map, SVG file, accessed August 2018, Available from URL: https://commons.wikimedia.org/wiki/File:Australia_medium-res_locator_map.svg, CC BY 4.0
- Tennessen JA & Zamudio KR. 2003. Early male reproductive advantage, multiple paternity and sperm storage in an amphibian aggregate breeder. *Molecular Ecology* **12**, 1567–1576.
- Teuscher M, Braendle M, Traxel V & Brandl R. 2009. Allometry between leg and body length of insects: lack of support for the size–grain hypothesis. *Ecological Entomology* **34**, 718–724.
- Torres-Vila LM, Rodríguez-Molina MC & Jennions MD. 2004. Polyandry and fecundity in the Lepidoptera: can methodological and conceptual approaches bias outcomes? *Behavioral Ecology and Sociobiology* **55**, 315–324.
- Young CJ. 2006. Molecular relationships of the Australian Ennominae (Lepidoptera: Geometridae) and implications for the phylogeny of the Geometridae from molecular and morphological data. *Zootaxa* **1264**, 1–147.
- Zahiri R, Kitching IJ, Lafontaine JD *et al.* 2011. A new molecular phylogeny offers hope for a stable family level classification of the Noctuoidea (Lepidoptera). *Zoologica Scripta* **40**, 158–173.

Accepted for publication 20 November 2019.