Different environments lead to a reversal in the expression of weapons and testes in the heliconia bug, *Leptoscelis tricolor* (Hemiptera: Coreidae)

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In many species, males invest both in weapons used in competitions for access to mates and testes size to increase competitive chances for fertilizations. The expression of weapons and testes can be sensitive to environmental factors experienced during development. However, relatively few studies have examined the effects of discrete, natural developmental environments on the expression of these traits in wild populations. In the present study, we examined the development of these primary and secondary sexual traits for the Heliconia bug, *Leptoscelis tricolor* (Hemiptera: Coreidae) across two different natural host plants, *Heliconia mariae* and *Heliconia platystachys*. Development on *H. platystachys* resulted in increased investment in weapons and reduced investment in testes, whereas development on *H. mariae* resulted in the opposite pattern. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, ••, ••–••.

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INTRODUCTION

Sexual selection has led to a striking diversity of weapons used in competition for reproductive opportunities. Early studies of sexual selection focused primarily on these conspicuous traits displayed during pre-copulatory male contests, with the underlying assumption that copulation inevitably leads to fertilization (Darwin, 1871; Huxley, 1932). It is now clear that copulation does not necessarily lead to fertilization, and competition among males can continue after mating in the form of sperm competition (Parker, 1970). Indeed, in many animal systems, traits that increase mating success and traits that increase fertilization success are both important for overall reproductive success (Preston *et al.*, 2003; Trillo, 2008; Eberhard, 2009; Rashed & Polak, 2009; Puniamoorthy, Blanckenhorn & Schäfer, 2012; Rahman, Kelley & Evans, 2013; Sbilordo & Martin, 2014).

Male sexually-selected weapons are used in combat or as signals to other males of the same species, and individuals bearing larger weapons often outcompete those with smaller weapons (Emlen, 2008). Males may also improve their fertilization success by investing in ejaculate traits, including ejaculates with more sperm (Parker, 1970, 1998; Parker & Pizzari, 2010). Selection for higher numbers of sperm can lead to larger testes size. Indeed, testes size correlates positively with levels of sperm competition across many taxa (Gage, 1994; Harcourt, Purvist & Liles, 1995; Byrne, Roberts & Simmons, 2002).

Although the expression of larger weapons and testes can be advantageous, the development of these traits and their resulting size is often dependent on environmental factors. Traits under strong directional selection, such as sexually-selected traits, are predicted to be especially responsive to environmental factors (Pomiankowski, 1987; Grafen, 1990; Cotton, Fowler & Pomiankowski, 2004). Many male sexually-selected traits are particularly phenotypically-plastic and environmentally-sensitive (Griffith, Owens & Burke, 1999; Moczek & Emlen, 1999; Qvarnström, 1999; Miller & Emlen, 2010b; Vergara *et al.*, 2012).

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Studies have shown that the expression of large secondary-sexual characters comes at a cost to other life-history traits such as immunity and dispersal ability (Hosken, 2001; Lewis, Price & Wedell, 2008; Nakayama & Miyatake, 2010; Yamane *et al*., 2010; Kelly, Neyer & Gress, 2014). Additional studies have shown differential investment in sexually-selected traits in response to social cues during development (Kasumovic & Brooks, 2011). However, relatively few studies have examined the effect of the natural environment on the investment in male pre- and post-copulatory traits. Most studies have been conducted in laboratory settings (Simmons & Emlen, 2006; Evans, 2010; Devigili *et al*., 2012; Pischedda & Rice, 2012; Rahman *et al*., 2013); thus, little is known about relationships among these traits under real-world environmental conditions (Kelly, 2008; Painting & Holwell, 2013). We expect that the relative expression of pre- and post-copulatory traits will vary plastically across space and time for natural populations as nutrition and social environments fluctuate. Some of this plasticity may be adaptive and some may not, although, in any case, this will likely have consequences for sexual selection, reproductive success, and even population persistence.

The present study aimed to examine the expression of weapons and testes as they occur in two distinct natural environments. According to life-history theory, differential allocation to fitness related traits should be more likely to occur in environments where resources are limited (van Noordwijk & de Jong, 1986; de Jong & van Noordwijk, 1992). Under these conditions, individuals cannot always acquire sufficient resources to maximize multiple traits (Reznick, Nunney & Tessier, 2000). Similarly, predictions from sperm competition theory suggest that investment in somatic pre-copulatory traits should come at a cost to investment in traits involved in sperm competition (Parker, 1998). In the present study, we investigated *Leptoscelis tricolor* Westwood (Hemiptera: Coreidae), a sexually dimorphic insect where males vary in their expression of sexual traits. We examined the expression of male weapon size and testes mass for insects collected in nature on two of their common host plants. We predicted that insects producing larger sexually-selected weapons will invest less in testes mass, based on the theory that males have a limited pool of resources from which to invest in pre- and post-copulatory reproductive traits and hence must invest differentially (Parker, 1998).

MATERIAL AND METHODS
STUDY ORGANISM

The Heliconia bug, *L. tricolor* Westwood (Hemiptera: Coreidae), provides an opportunity to examine the

effects of alternate environments on male sexually-selected traits. Some insects in this family possess enlarged hind legs and, in many species, males with larger hind femurs are more likely to win competitions (Mitchell, 1980; Miyatake, 1997; Eberhard, 1998; Procter, Moore & Miller, 2012). *Leptoscelis tricolor* are sexually dimorphic. Males exhibit enlarged hind femurs with spines, and use these weapons to squeeze their male opponents in combat (Fig. 2; Miller, 2008; Miller & Emlen, 2010a, b). Mating occurs when a male taps his forelegs and waves his antennae in front of a female, after which the male mounts the female and attempts to establish contact with her genitalia. A female must open a genital sclerite to allow for intromission; thus, males are not always successful in mating. However, once intromission is achieved, the male faces away from the female and the pair remains in copula, often for extended periods of time. Male and female *L. tricolor* mate multiply and copulation often lasts multiple hours (Miller, 2008). Although sperm competition has not yet been examined in this species, sperm competition has been recorded in other Hemiptera (Rubenstein, 1989; Carroll, 1991).

The life-history of *L. tricolor* is tightly linked to the inflorescences of heliconia plants (Zingiberales: Heliconiaceae). *Leptoscelis tricolor* feed, mate, and lay eggs on heliconia inflorescences, and mark–recapture studies reveal that adults commonly visit multiple heliconia species during their lifetimes (Miller, 2007). By contrast, juvenile *L. tricolor* are wingless and their growth and development is usually limited to the inflorescence on which they hatch (Miller & Hollander, 2010; Miller & Emlen, 2010b). Hemimetabolous insects, such as *L. tricolor*, do not grow in body size after reaching adulthood; once these insects undergo their final molt to adulthood, their exoskeleton becomes sclerotized and their external morphology remains much the same throughout their adult life (Miller & Emlen, 2010b).

In Panama, *L. tricolor* can frequently be found on *Heliconia mariae* and *Heliconia platystachys*. Previous research has established that insects maturing on *H. platystachys* when it is blooming and fruiting develop larger bodies and hind femurs than insects that mature on *H. mariae* plants at the same phenological stage (Miller & Emlen, 2010b). Although inherent nutritional differences in the host plants are probably responsible for the differences in growth, the distinct microhabitat structures (Fig. 1A, B) and biotic communities provided by each plant species may be influential. For example, if predator densities are higher on one host species, insects may spend more time hiding and less time feeding. Additionally, the conspecific social environment may influence
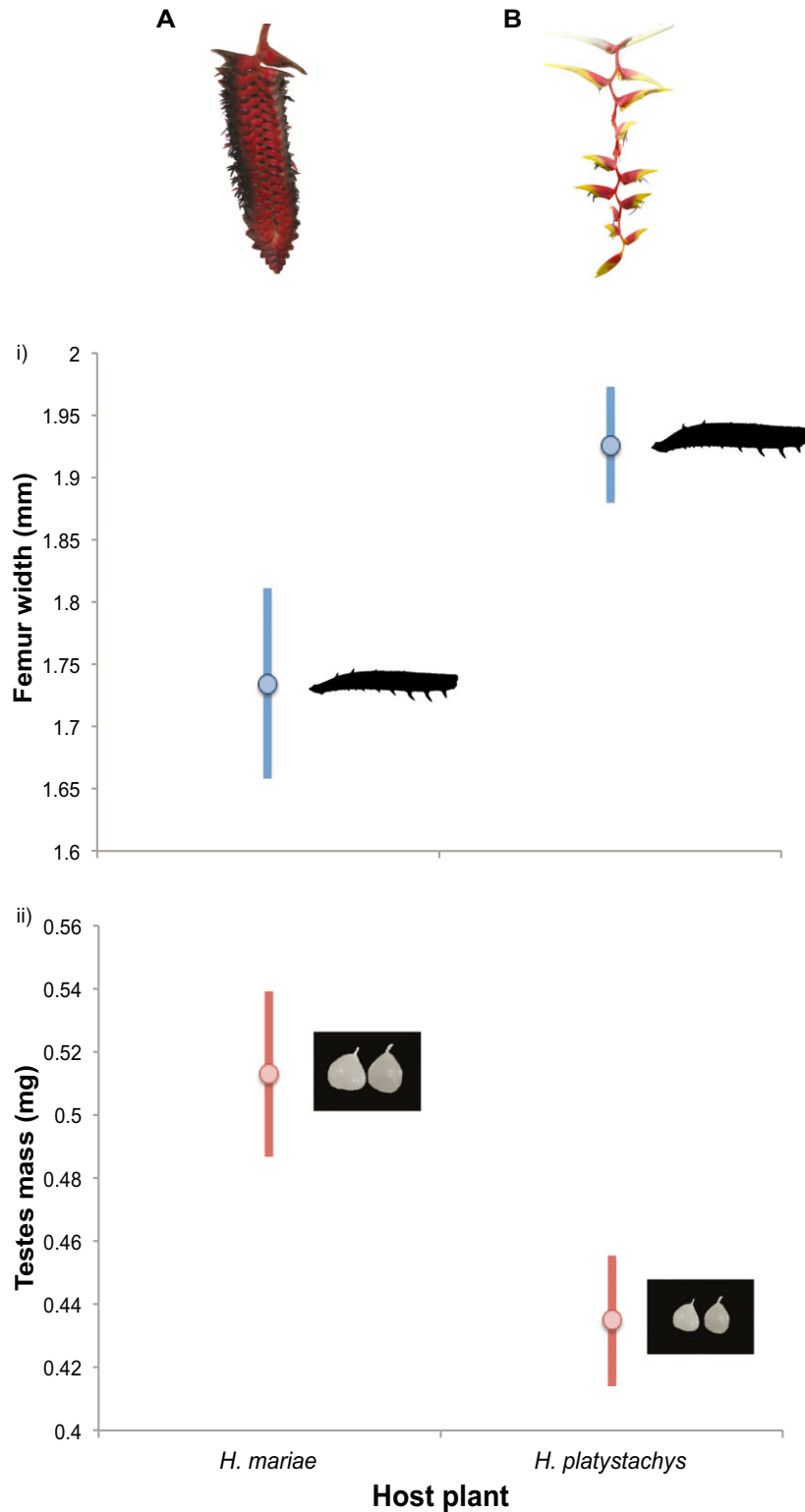
Figure 1. Inflorescences belonging to *Heliconia mariae* (A) and *Heliconia platystachys* (B). (i) Male *Leptoscelis tricolor* raised on host plant *H. mariae* exhibited smaller hind femurs (mean ± SE: 1.73 ± 0.07 mm, *N* = 18) compared to *H. platystachys* where males exhibited larger femurs (1.92 ± 0.05 mm, *N* = 34, Cohen's d = 0.70). (ii) Males raised on *H. mariae* have heavier testes (0.51 ± 0.03 mg, *N* = 18) than those raised on *H. platystachys* (0.44 ± 0.04 mg, *N* = 35, Cohen's d = 0.70).

Figure 2. Male *Leptoscelis tricolor* displaying enlarged hind femurs used in male–male competition (photograph by P. Marting, Arizona State University, Tempe, AZ, USA).

allocation to weapons and testes as seen in other species (Kasumovic & Brooks, 2011).

Host plants and insect rearing

All data were collected within an area of 25 km² near Gamboa, Panama in July and August 2013. We found Heliconia inflorescences by visually scanning the forests around Gamboa. We located insects on inflorescences belonging to *H. platystachys* and *H. mariae* of similar phenological stage (i.e. with fruit and flowers). Fine mesh bags were slipped over individual inflorescences where juveniles in the fourth and fifth instar were found. Only one juvenile was enclosed per bag; any additional nymphs were transferred to another host plant. We returned 26 days later (for juveniles found as fourth instars) and 19 days later (for juveniles found as fifth instars) to collect sexually mature adults estimated to be on average 17 days old (± 5 days) and immediately placed them in individual deli cups with moist paper towels.

Weapon measurements and testes weights

Male pronotal width and hind femur width were measured using Mitutoyo digital calipers (maximum accuracy 0.01 mm). Pronotal width is a common metric of body size in insects and is highly correlated with overall body size in this insect family (Gillespie *et al.*, 2014). We measured pronotal width at the widest point. Hind femur widths were measured on the third distal spine, which represents the widest part of the femur and the part that most likely contacts opponents during competitions (Miller & Emlen, 2010a, b). Within 24 h of removal from the field, males were dissected under saline solution (0.09% NaCl) and testes were extracted and placed on pre-weighed pieces of aluminum foil. Testes samples were placed in a drying oven at 60 ± 3 °C and after a

minimum of 5 days in the drying oven, were weighed to a precision of 0.01 mg within 6 min of extraction from the drying oven.

RESULTS

We found that males raised on *H. platystachys* were larger in body size than males raised on *H. mariae* [mean ± SE: *H. platystachys* = 7.04 ± 0.08 mm, N = 34; *H. mariae* = 6.75 ± 0.11, N = 18; analysis of variance (ANOVA): $F_{1,50}$ = 4.25, P = 0.044]. Similarly, weapon size was larger on *H. platystachys* than on *H. mariae* (ANOVA: $F_{1,50}$ = 5.46, P = 0.024). By contrast, males raised on *H. mariae* had a greater testes mass than males raised on *H. platystachys* (ANOVA: $F_{1,51}$ = 5.90 and P = 0.019; Fig. 1).

Some males reared on *H. platystachys* were estimated to be 12 days old (N = 11), whereas all other males raised on *H. platystachys* (N = 24) and all males raised on *H. mariae* (N = 18) were older than 14 days. To ensure the observed difference in testes mass was not a result of age differences, we analyzed the effect of age on testes size for the subset of males reared on *H. platystachys*. We found no evidence that age influenced testes mass (ANOVA: $F_{1,32}$ = 1.13 and P = 0.295).

DISCUSSION

The present study reveals that ecologically relevant variation in developmental environment modifies the phenotypic expression of both weapons and testes, which are traits that likely function in pre- and post-copulatory sexual selection in *L. tricolor*. We found that *L. tricolor* reared on the host plant *H. platystachys* develop larger body and weapon size but have smaller testes than insects reared on *H. mariae*. Consistent with previous work on this sexually dimorphic insect, we found that the size of males and the size of weapons used in pre-copulatory contests differed depending on the host plant on which males developed (Miller & Emlen, 2010b). Other studies have shown similar effects of developmental environment on weapon size (Miller & Emlen, 2010b; Emlen *et al.*, 2012), testes mass, and sperm production (Gage & Cook, 1994; Simmons & Kotiaho, 2002). These results show an inverse relationship in the expression of weapons and testes in this population across two common developmental environments.

The idea that individuals have a limited pool of resources from which to allocate to fitness-related traits is the basic premise of the concept of trade-offs (Stearns, 1987). Potential allocation trade-offs have been found between male sexually-selected traits and other life-history traits such as immunity (Lewis *et al.*, 2008), dispersal ability (Yamane *et al.*, 2010),

and predation avoidance (Nakayama & Miyatake, 2010). Evidence of allocation trade-offs between pre- and post-copulatory traits is mixed (evidence for trade-offs: Moczek & Nijhout, 2004; Simmons & Emlen, 2006; Yamane *et al.,* 2010) (no trade-offs found: Devigili *et al.*, 2012; Painting & Holwell, 2013; Rahman *et al.*, 2013). Indeed, life-history theory predicts that individuals in excellent environments or those with a heightened ability to acquire resources from the environment may be able to optimally express multiple traits (van Noordwijk & de Jong, 1986; de Jong & van Noordwijk, 1992; Reznick *et al.*, 2000). Most studies examining the relationships between pre- and post-copulatory traits have been conducted under laboratory conditions (but see also Kelly, 2008; Painting & Holwell, 2013), where natural environmental contexts are often difficult to reconstruct. The present study, in contrast, focused on a wild population of insects. Although the striking pattern found here suggests an allocation trade-off in expression (Fig. 1), additional studies are required to fully test this hypothesis (Zera & Harshman, 2001; Tomkins & Simmons, 2002). Future studies might also examine the role that selection plays in driving the observed patterns in expression of testes and weapons.

Plasticity in the development of pre- and post-copulatory traits can allow individuals to match the competitive context that they may encounter at maturity (Kasumovic & Brooks, 2011). In *L. tricolor,* male–male contest competition occurs on heliconia inflorescences and competition may differ depending on the species of heliconia (Miller, 2007; Somjee, 2014). For example, more *L. tricolor* males with larger hind femora were found on *H. platystachys* compared to *H. mariae* (Miller & Emlen, 2010a). Thus, a *L. tricolor* nymph may use its developmental host plant as a cue to predict future intraspecific competition in this context or for the time of year, and allocate investment accordingly. A recent model by Parker, Lessells & Simmons (2013) explicitly addresses the fitness pay-off associated with investment in pre- versus post-copulatory traits under different levels and types of male competition. One prediction of this model is that males that experience contest competition will maximize their fitness returns by allocating preferentially more to weapons as the competition intensity (number of males competing for a single mate) increases (Parker, Lessells & Simmons, 2013). Based on our results and predictions from Parker *et al*. (2013), we would predict that contest competition may be more important for fitness on *H. platystachys* (where males preferentially invest in weapons) compared to *H. mariae* (where males preferentially invest in testes). Alternatively, environmental factors such as nutrition that are not associ- ated with competition type or intensity may be responsible for the patterns found and additional research is required to understand the precision by which selection can act to affect the relationships between these traits.

Other studies have found that different developmental environments can lead to functionally significant differences in the size and shape of male sexual traits (Worthington, Berns & Swallow, 2012). Developmental responses to different ecological conditions produce trait variation and can lead to selection and subsequent genetic accommodation of these plastic phenotypes (West-Eberhard, 2005; Rodríguez & Al-Wathiqui, 2012). One of the most exciting topics in the field of sexual selection today is the role of ecological complexity in producing and maintaining variation in male sexual traits (Miller & Somjee, 2014; Miller & Svensson, 2014). The potential for pre- and post-copulatory traits to vary across environments offers opportunities to investigate the role of the environment on the evolution of mating behaviour (Emlen, 1997; Goos, Cothran & Jeyasingh, 2014), sexual dimorphism (Baeza & Asorey, 2012), and species divergence (Elzinga, Mappes & Kaila, 2014).

Our results demonstrate that the host plant environment affects the expression of both pre- and post-copulatory traits in *L. tricolor*. However, because we do not know the mechanism by which environments affect changes in the expression of weapons and testes, we cannot specify why the observed pattern exists. Nevertheless, the finding in the present study that the expression of weapons and testes shows a negative phenotypic relationship across host plant species highlights the importance of measuring pre- and post-copulatory sexual traits in the wild across natural environmental contexts. The potential for environmentally induced variation to affect relationships between sexually-selected traits provides an exciting avenue for understanding the role that the environment plays in the diversity of sexual traits in nature.

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