SHORT COMMUNICATION

Sexual selection and the evolution of secondary sexual traits: sex comb evolution in *Drosophila*

RHONDA R. SNOOK*, NELLY A. GIDASZEWSKI*§, TRACEY CHAPMAN† & LEIGH W. SIMMONS‡

*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
†School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, UK
‡School of Animal Biology (M092), Centre for Evolutionary Biology, The University of Western Australia, Crawley, Australia

Abstract

Sexual selection can drive rapid evolutionary change in reproductive behaviour, morphology and physiology. This often leads to the evolution of sexual dimorphism, and continued exaggerated expression of dimorphic sexual characteristics, although a variety of other alternative selection scenarios exist. Here, we examined the evolutionary significance of a rapidly evolving, sexually dimorphic trait, sex comb tooth number, in two *Drosophila* species. The presence of the sex comb in both *D. melanogaster* and *D. pseudoobscura* is known to be positively related to mating success, although little is yet known about the sexually selected benefits of sex comb structure. In this study, we used experimental evolution to test the idea that enhancing or eliminating sexual selection would lead to variation in sex comb tooth number. However, the results showed no effect of either enforced monogamy or elevated promiscuity on this trait. We discuss several hypotheses to explain the lack of divergence, focussing on sexually antagonistic coevolution, stabilizing selection via species recognition and nonlinear selection. We discuss how these are important, but relatively ignored, alternatives in understanding the evolution of rapidly evolving sexually dimorphic traits.

Introduction

Darwin recognized that the existence of marked sexual dimorphism in 'display' traits, one of the most striking, frequent and easily observed of natural phenomena, represented a potential problem for his theory of natural selection. However, he also saw a powerful resolution – that individuals not only struggle for existence but also compete to acquire mates. He realized that this opportunity for selection can often result in the evolution of sex-limited traits that function in competing for, or choosing, mates (Darwin, 1871). Thus, sexual dimor-

phism, particularly in secondary sexual traits, is considered to be a hallmark of sexual selection. In males, secondary sexual traits can take the form of armaments or ornaments, and their presence and degree of expression are often correlated with a male's mating success (Andersson, 1994). Secondary sexual traits can take a huge variety of forms, such as morphological traits including the antlers of deer and horns of beetles, or traits that function in communication and signalling such as the acoustic signals of birds and insects. One consistent pattern across taxa is that such traits tend to evolve very rapidly (Andersson, 1994). This has led to the idea that there is strong sexual selection acting upon sexually dimorphic traits that function in sexual display.

Further opportunities for selection can arise if there is sexual conflict, that is, if male and female fitness interests diverge (Parker 1979). This conflict will be exacerbated in promiscuous mating systems in which breeding pairs are unrelated to one another. Under this

Correspondence: Rhonda R Snook, Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK S10 2TN. Tel.: +44 114 222 0126; fax: +44 114 222 0002;
e-mail: r.snook@sheffield.ac.uk
§Present address: Département Systématique et Evolution Muséum National d'Histoire Naturelle UMR 7205 OSEB 45 rue Buffon 75005 Paris France

scenario, strategies employed by males that enhance their reproductive success, but at cost to their current mate can nevertheless be strongly selected. Such conflict may promote the evolution of sexually antagonistic traits in one sex and counter adaptations in the other. Both verbal and mathematical models of sexually antagonistic coevolution suggest that males are more likely to evolve multiple, rather than exaggerated single, manipulative traits. Hence, males are expected to harbour a 'mosaic of newer (more effective) and older (less effective) traits' (Rice & Holland, 1997; Holland & Rice, 1998; Moore & Pizzari, 2005). There is then the possibility that the 'less effective' traits that are retained function in stimulating females, but are no longer subject to sexual selection.

In addition to the forces described above, sexually dimorphic traits may also be shaped by natural selection for mate recognition. Such selection could prevent or limit gene flow between populations and species (e.g. Ryan & Rand, 1993; Pfennig & Pfennig, 2009 but see Mendelson & Shaw, 2012). Hence, directional sexual selection for trait elaboration could be countered by stabilizing selection for mate/species recognition. This pattern of selection could be evident as reproductive character displacement (e.g. Martin *et al.*, 2010; Pfenning & Hurlbert, 2012).

One example of a rapidly evolving sexually dimorphic trait potentially subject to divergent opportunities for selection discussed above is the male sex combs of Drosophila fruitflies. These structures are a set of modified mechanosensory bristles found on the front legs of males and which are not present in females. Sex combs have evolved relatively recently and are present only in the subgenus *Sophophora*, which is comprised of the *melanogaster* and *obscura* group species (Lakovaara & Saura, 1982; Lemeunier *et al.*, 1986). The sex combs in Sophophorans can be primitively absent or secondarily lost; however, when present, their morphology is strikingly diverse. There is significant interspecific variation in the position of the sex comb (on tarsus 1, or tarsus 2, or both), its orientation on the tarsus (rotated or transverse), the number of bristles or 'teeth' on the comb (2–44) and the size, shape and colour of the teeth (Kopp & True, 2002; Barmina & Kopp, 2007). Given this variability, sex comb evolution has been described as one of 'the most remarkable changes in drosophilids worldwide' (Lachaise & Chassagnard, 2002). Reconstruction of ancestral sex comb character states of 46 species of the *melanogaster* and *obscura* species groups using Bayesian inference confirmed rapid evolution in both rotation and position of the sex comb (Barmina & Kopp, 2007), although as yet no formal analysis has been performed on sex comb tooth number.

The sex combs function during mating and the dramatic variation in sex comb morphology is thought to be driven primarily by directional sexual selection (e.g. Kopp & True, 2002; Polak & Starmer, 2005; Polak &

Tomkins, 2012; Ng & Kopp, 2008; Spieth, 1952; Cook, 1977; Coyne, 1985; Markow *et al.*, 1996; Polak *et al.*, 2004; Ahuja & Singh, 2008; Ng & Kopp, 2008). Several species have been studied with respect to sex comb function. In this study, we focus on two: *Drosophila melanogaster* and *Drosophila pseudoobscura*. In these species, there is a similar orientation of the sex combs (rotated), but their position (tarsus 1 for *D. melanogaster* and tarsus 1 and 2 for *D. pseudoobscura*) and the total number of teeth for each sex comb differ. Likewise, the function of the sex comb during mating differs. In *D. pseudoobscura*, the sex combs appear to provide traction while the male is spreading the female's wings to mount successfully (Spieth, 1952). In contrast, in *D. melanogaster*, sex combs function in grasping the female's external genitalia immediately prior to mounting (Cook, 1977).

Several studies in these two species have tested for associations between male mating success and the presence/absence of sex combs (Spieth, 1952; Cook, 1977; Ng & Kopp, 2008) by physical (Spieth, 1952; Cook, 1977) or genetic (Ng & Kopp, 2008) sex comb ablation. These studies found that the presence of the sex comb is necessary for males to successfully mate. The impact of sex comb tooth number on male mating success has been tested by artificial selection (Ahuja & Singh, 2008) and by exploiting natural variation in wild populations (Markow *et al.*, 1996). However, no relationship between the number of sex comb teeth and male mating success has yet been detected (Markow *et al.*, 1996; Ahuja & Singh, 2008). This presents a paradox because these species otherwise have all the hallmarks of a trait whose evolution is driven by sexual selection (i.e. they are sexually dimorphic, show rapid divergence and have a functional relationship with copulation). Hence, it appears that either the adaptive value or functional significance of sex comb tooth number has not yet been correctly identified.

Techniques previously used to assess the relationship between sex comb tooth number and mating success may not have been able to capture with sufficient resolution the role of sexual selection for three reasons. First, if sex combs are selected via female preference, then artificial selection on sex comb tooth number *per se* would not necessarily result in changes in female preference. Hence, tests on lines artificially selected for sex comb tooth number may not detect any association between male mating success and the trait (Ahuja & Singh, 2008) because female preference is not coselected. Second, tests on traits in wild populations (Markow *et al.*, 1996) may fail to detect selection if the relevant traits are at or near equilibrium (Endler, 1986). With respect to studies of natural populations, evidence that a trait evolves in response to sexual selection requires observation of a cause-and-effect relationship between the trait and the sexual-selection environment. Only a few methods, for example, the perturbation of the natural environment, can reliably establish such rela-

tionships (Endler, 1986). In this case, such perturbation could take the form of changes in the intensity of sexual selection. Third, either or both pre and post-mating (*Drosophila bipectinata:* Polak & Simmons, 2009) selection may drive sex comb tooth number evolution; however, most studies test for associations between sex comb tooth number and male mating success, and thus ignore post-copulatory selection.

Experimental evolution is one technique that can address all of the experimental constraints described above. In this method, the sexual selection environment (i.e. intensity) in which the focal and other coevolving traits, including female preference, interact is modified. Manipulation of the sexual-selection environment can include both pre and post-copulatory selection and perturbs the focal trait from an equilibrium state. In this study, we used experimentally evolved populations of *D. melanogaster* and *D. pseudoobscura* in which the opportunity for sexual selection was manipulated by either completely eliminating (via enforced monogamy) or strongly elevating (via biasing the sex ratio towards more males than females) the intensity of sexual selection. This environment manipulated both pre and post-copulatory sexual selection and likewise allowed for coevolution between male and female traits. Previous work on the two species used here has demonstrated that experimental sexual selection can modify traits predicted to be driven by both pre and post-mating sexual selection (Wigby & Chapman, 2004; Crudgington *et al.*, 2005, 2009, 2010; Snook *et al.*, 2005; Reuter *et al.*, 2008; Wigby *et al.*, 2009).

Using these lines, we tested the hypothesis that sexual selection drives sex comb tooth number evolution and predicted that populations experiencing different intensities of sexual selection should exhibit divergence in this trait. At present, the direction of change is difficult to predict because studies in other *Drosophila* species report that mating success may be higher in individuals with either fewer sex comb teeth (e.g. *Drosophila simulans*, Markow *et al.*, 1996) or with more teeth (e.g. *D. bipectinata*, Polak *et al.*, 2004).

Materials and methods

Experimental evolution

Drosophila melanogaster
Opportunities for sexual selection were manipulated by establishing from the Dahomey wild-type stock three replicate lines each of three experimental evolution treatments: (1) female biased (FB, 25 males and 75 females), (2) equal sex (ES, 50 males and 50 females) and (3) male biased (MB, 75 males and 25 females). The establishment and maintenance of these lines has previously been described in full in Wigby & Chapman (2004). The sex ratio in the MB lines was changed to 70 males and 30 females from generation 53 to ease propagation by increasing the number of females. Flies were provided access to water and were fed *ad libitum* with two vials of sugar-yeast food (Bass *et al.*, 2007) with added live yeast every 2 or 3 days. Nine days after the cages were set up, eggs were collected. The majority of eggs were allowed to hatch before larvae were collected, thus minimizing selection on early egg hatchability. Larvae were raised at standard density (100/vial) to minimize environmentally determined differences in adult body size. All adults were allowed to eclose over 2 days (i.e. were not virgin, to avoid selection on development time) before sorting them into their appropriate sex-ratio treatments. The lines were selected for about 70 generations until 2007, and then maintained at 18 °C (to slow down generation times by a factor of 2) until March 2008 when a further 26 generations of selection were applied until May 2010. Thus, the total number of generations at the time of ethanol preservation for subsequent sex comb analysis was 110, with the total number of generations of selection being 96.

Drosophila pseudoobscura
Opportunities for sexual selection were manipulated by establishing four replicate lines of each of three experimental evolution treatments: (1) enforced monogamy (M: one female and one male), (2) intermediate promiscuity (C: one female and three males) and (3) elevated promiscuity (E: one female and six males). In this experiment, we examined only three replicates. The establishment and maintenance of these lines has previously been described in full in Crudgington *et al.* (2005, 2009). Lines have been maintained under continuous selection. Importantly, we have successfully controlled for variation in effective population size by increasing the number of families in the M treatment relative to E (Bacigalupe *et al.*, 2008; Snook *et al.*, 2009). Experimental flies from the three sexual-selection treatments were generated from different generations depending on the replicate (generations 45–51 of replicate 1 (see Snook *et al.*, 2010); generation 62 for replicate 3 and generation 60 for replicate 4) by using standard densities of 100 first instar larvae per food vial (Crudgington *et al.*, 2009).

Sex comb measurement and body size

Males from each treatment, replicate, and species were preserved separately in ethanol until measurements were made. We measured sex comb tooth number by removing both front legs (right and left) and mounting them on a glass slide in a drop of glycerol. The number of teeth for each comb was counted and recorded separately, using a compound microscope at a magnification of 400 ×. We determined body size in *D. melanogaster* as the length of the tibia, measured using a compound microscope at a magnification of 200 × (Robertson,

1962). For *D. pseudoobscura,* we measured wing vein length IV (WVL) as previously described (Robertson, 1962; Crudgington *et al.*, 2009).

Statistical analysis

The unit of replication in experimental evolution studies is the number of replicate lines for each treatment. Thus, we used ANOVAS to determine whether sexual-selection regime influenced the number of sex comb teeth by nesting replicate lines within treatment and including body size as a random effect. These analyses provided the appropriate degrees of freedom, and avoided pseudoreplication associated with measuring multiple flies per replicate line. All analyses were conducted using JMP v. 7.

Results and discussion

The mean (±SE) number of sex comb teeth across all replicates for each treatment for each species is reported in Table 1. For *D. melanogaster*, we found no evolutionary divergence in the number of sex comb teeth due to sexual-selection treatment (Table 2). Larger males tended to have a greater number of sex comb teeth, and there was a significant random effect of replicate nested within treatment (Table 2). For *D. pseudoobscura*, there was again no effect of sexual selection on sex comb tooth number, but significant variation among replicates (Table 3). There was no effect of body size on tooth number.

Here, we tested the hypothesis that a rapidly evolving, secondary sexual trait was being significantly influenced by sexual selection, in two different species each with contrasting sex comb morphologies and functions. We resolved some of the potential technical problems of previous studies by using experimental evolution in which the intensity of sexual selection was altered, and predicted that populations experiencing different levels of sexual selection should differ in sex comb tooth number. However, in both species, we found no effect of sexual-selection treatment on sex comb tooth number, indicating that this structure is not currently being driven by sexual selection in either species. Several alternative hypotheses could explain the lack of divergence in sex comb tooth number.

First, heritable variation in these populations may be low. However, this seems highly unlikely, because we observed substantial phenotypic variation in sex comb tooth number in the replicates (Tables 2 and 3). In addition, for each species, other traits connected to pre and post-mating success have diverged predictably in these same experimentally evolved populations (Wigby & Chapman, 2004; Crudgington *et al.*, 2005, 2009, 2010; Snook *et al.*, 2005; Reuter *et al.*, 2008; Wigby *et al.*, 2009).

Table 1 Mean number (±SE) of teeth in the combined left and right sex combs of Drosophila species derived from populations subjected to experimental evolution under varying sexual-selection intensities.

Species			Number of teeth
Drosophila melanogaster			
Female biased			22.0 ± 0.87
Equal sex ratio			21.8 ± 0.8
Male biased			21.4 ± 0.8

	Top	Bottom	Total
Drosophila pseudoobscura			
Monogamous	12.8 ± 0.6	11.1 ± 0.5	24.5 ± 0.9
Intermediate promiscuity	13.1 ± 0.5	11.4 ± 0.5	23.7 ± 0.8
Elevated promiscuity	12.6 ± 0.5	11.1 ± 0.4	23.9 ± 1.0

Table 2 Nested ANOVA performed on the total number of teeth on the sex combs of *Drosophila melanogaster* evolving under different intensities of sexual selection (treatment) with treatment nested within replicate and tibia length to control for body size.

Effect	MS	d.f.	F	P
Treatment	22.02	2	0.22	0.811
Replicate [Treatment]	102.4	6	49.85	< 0.001
Tibia length	11.12	1	5.41	0.021
Error	2.05	439		

Table 3 Nested ANOVA performed on the total number of teeth on the sex combs of *Drosophila pseudoobscura* evolving under different intensities of sexual selection (treatment) with treatment nested within replicate and wing length to control for body size.

Effect	MS	d.f.	F	P
Treatment	21.82	2	0.21	0.82
Replicate [Treatment]	117.43	6	57.8	< 0.001
Wing length	0.37	1	0.18	0.672
Error	2.03	429		

Second, sex comb tooth number may be evolutionarily constrained either by physiochemical, genetic and developmental factors (Wagner, 2011), or by trade-offs. The HOX gene, *sex combs reduced* (*Scr*), controls the presence/absence of the sex comb (Struhl, 1982; Barmina & Kopp, 2007) and may also control the number of sex comb teeth that are formed (Kaufman *et al.*, 1980; Lewis *et al.*, 1980; Pattatucci *et al.*, 1991). Despite divergence in sex comb position and tooth number, both species share the same developmental mechanism for sex comb formation (Barmina & Kopp, 2007; Tanaka *et al.*, 2009); thus, the lack of response to selection could be attributable to shared developmental and genetic constraints. Trade-offs in investment in other sexually selected traits could also limit the response to

selection. Such trade-offs in these experimentally evolved populations have not yet been studied.

A third explanation for the lack of a response to selection is that stabilizing, rather than directional, selection may influence current evolution of sex combs. When sexually dimorphic structures, like sex combs, are useful for taxonomic classification, then they may also be candidates for species recognition (e.g. Ryan & Rand, 1993; Pfennig & Pfennig, 2009; McPeek et al., 2010). Two alternative outcomes are possible: (1) trait exaggeration continues when either directional selection enhances female preference variation between species through benefits accrued via sexual selection or through natural selection against hybridization, or (2) stabilizing selection on the trait occurs when females have overlapping preferences for con- and hetero-specific males (Pfennig, 1998), subsequently reducing female preference (Pfennig 2000). The extent to which traits are driven and limited by both sexual selection and speciation is of general interest, given that secondary sexual traits can function in both contexts. Artificial selection on *D. melanogaster* sex comb tooth number indicated that stabilizing selection may be acting on this trait (see Ahuja & Singh, 2008). However, if sex comb structure was under stabilizing selection to facilitate species recognition, then the trait should vary little among populations across the species' range (Butlin, 1995a), yet *D. melanogaster* stock populations exhibit substantial variation in sex comb tooth number (Ahuja & Singh, 2008). Reproductive character displacement is also predicted under this scenario (Howard, 1993; Butlin, 1995b; Servedio & Noor, 2003; Pfennig & Pfennig, 2009) but, to our knowledge, no studies have examined this. Thus, the extent to which sex comb tooth number is being driven by stabilizing selection is unknown.

Another limitation to directional divergence in traits is nonlinear selection. A recent field study of *D. melanogaster* found evidence of disruptive selection on sex comb tooth number (Robinson et al., 2012). Tooth number in wild populations had no impact on mating success, but it did influence fertilization success, with fitness peaks at both low and high tooth numbers. Such disruptive selection can generate considerable among-population variation (e.g. as in Ahuja & Singh (2008), and can limit directional divergence in evolving lines.

Sexually antagonistic coevolution is another explanation for the lack of response to current sexual selection. Sex comb tooth number could represent a relic of past selection with no current role in determining competitive reproductive success, but be required nevertheless to provide female courtship stimulation. Previous studies for other sexually dimorphic traits have shown that male mating success is not influenced by an exaggerated trait but that in unselected populations such exaggeration increases male fitness; this has been interpreted as suggesting a history of past conflict (e.g. McClintock & Uetz, 1996). Consistent with this hypothesis, our experimental populations of both species have experienced sexual conflict (Wigby & Chapman, 2004; Crudgington et al., 2005, 2010) and, in artificial selection studies, the deliberate exaggeration of *D. melanogaster* sex comb tooth number did not improve male fitness (Ahuja & Singh, 2008). However, the extent to which sex combs specifically, and sexually dimorphic traits generally, represent a sexual conflict 'graveyard' is unknown.

While here we have focussed on a rapidly evolving secondary sexual trait in Drosophila, the evolution of such traits in general is of wide interest to evolutionary biologists. The default assumption is that these structures function in directional sexual selection. However, a variety of alternative hypotheses, such as past sexual conflict, stabilizing selection for species recognition and nonlinear selection, may impact the current evolution of such structures. Future work in this and other systems with striking, rapidly evolving secondary sexual traits should consider these alternatives to gain a better understanding of the drivers and limitations of rapid evolution.

Acknowledgments

References

Ahuja, A. & Singh, R.S. 2008. Variation and evolution of male sex combs in Drosophila: nature of selection response and theories of genetic variation for sexual traits. *Genetics* **179**: 503–509.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

Bacigalupe, L., Crudgington, H.S., Slate, J., Moore, A.J. & Snook, R.R. 2008. Sexual selection and interacting phenotypes in experimental evolution: a study of *Drosophila pseudoobscura* mating behaviour. *Evolution* **62**: 1804–1812.

Barmina, O. & Kopp, A. 2007. Sex-specific expression of a HOX gene associated with rapid morphological evolution. *Dev. Biol.* **311**: 277–286.

Bass, T.M., Grandison, R.C., Wong, R., Martinez, P., Partridge, L. & Piper, M.D.W. 2007. Optimization of dietary restriction protocols in *Drosophila*. *J. Gerontol. A Biol. Sci. Med. Sci.* **62**: 1071–1081.

Butlin, R.K. 1995a. Genetic variation in mating signals and responses. In: *Speciation and the Recognition Concept* (M.D. Lambert & G.H. Spencer, eds), pp. 327–366. The Johns Hopkins University Press, Baltimore, Maryland.

Butlin, R.K. 1995b. Reinforcement: an idea evolving. *Trends Ecol. Evol.* **10**: 432–434.

Cook, R.M. 1977. Behavioral role of the sexcombs in *Drosophila melanogaster* and *Drosophila simulans*. *Behav. Genet.* **7**: 349–357.

Coyne, J.A. 1985. Genetic studies of three sibling species of Drosophila with relationship to theories of speciation. *Genet. Res.* **46**: 169–192.

Crudgington, H.S., Beckerman, A.P., Brüstle, L., Green, K. & Snook, R.R. 2005. Experimental removal and elevation of sexual selection: does sexual selection generate manipulative males and resistant females? *Am. Nat.* **165**: S72–S87.

Crudgington, H.S., Fellows, S., Badcock, N.S. & Snook, R.R. 2009. Experimental manipulation of sexual selection promotes greater mating capacity but does not alter sperm investment. *Evolution* **63**: 926–938.

Crudgington, H.S., Fellows, S. & Snook, R.R. 2010. Increased opportunity for sexual conflict promotes harmful males with elevated courtship frequencies. *J. Evol. Biol.* **23**: 440–446.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. John Murray, London.

Endler, J.A. 1986. *Natural Selection in the Wild*, 2nd edn. Princeton University Press, Princeton, NJ.

Holland, B. & Rice, W.R. 1998. Chase-away sexual selection: antagonistic seduction vs. resistance. *Evolution* **52**: 1–7.

Howard, D.J. 1993. Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. In: *Hybrid Zones and the Evolutionary Process* (R. Harrison, ed.), pp. 46–69. Oxford University Press, New York.

Kaufman, T.C., Lewis, R. & Wakimoto, B. 1980. Genetic analysis of chromosome 3 in Drosophila melanogaster: the homeotic gene complex in polytene chromosome interval 84A-B. *Genetics* **94**: 115–133.

Kopp, A. & True, J.R. 2002. Evolution of male sexual characters in the oriental Drosophila melanogaster species group. *Evol. Dev.* **4**: 278–291.

Lachaise, D. & Chassagnard, M.T. 2002. Divergence of sex comb phenotypes in the *Drosophila fima* species group and radiation on Afrotropical Ficus, including five new species from east Africa and Madagascar (Diptera: Drosophilidae). *Annales de la Société entomologique de France* **38**: 79–99.

Lakovaara, S. & Saura, A. 1982. Evolution and speciation in the *Drosophila obscura* group. In: *The Genetics and Biology of Drosophila*, vol. **3b** (M. Ashburner, H.L. Carson & J.N. Thompson Jr, eds), pp 1–59. Academic Press, London.

Lemeunier, F., David, J., Tsacas, L. & Ashburner, M. 1986. The melanogaster species group. In: *The Genetics and Biology of Drosophila*, vol. **3e** (M. Ashburner, H.L. Carson & J.N. Thompson Jr, eds), pp. 147–256. Academic Press, London.

Lewis, R.A., Kaufman, T.C., Denell, R.E. & Tallerico, P. 1980. Genetic analysis of the Antennapedia gene complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaster* I. Polytene chromosome segments 84B–D. *Genetics* **95**: 367–381.

Markow, T.A., Bustoz, D & Pitnick, S. 1996. Sexual selection and a secondary sexual character in two Drosophila species. *Anim. Behav.* **52**: 759–766.

Martin, P.R., Montgomerie, R. & Lougheed, S.C. 2010. Rapid sympatry explains greater color pattern divergence in high latitude birds. *Evolution* **64**: 336–347.

McClintock, W.J. & Uetz, G.W. 1996. Female choice and pre-existing bias: visual cues during courtship in two *Schizocosa* wolf spiders (Araaneae: Lycosidae). *Anim. Behav.* **52**: 167–181.

McPeek, M.A., Symes, L.B., Zong, D.M. & McPeek, C.L. 2010. Species recognition and patterns of population variation in the reproductive structures of a damselfly genus. *Evolution* **65**: 419–428.

Mendelson, T.C. & Shaw, K.L. 2012. The (mis)concept of species recognition. *Trends Ecol. Evol.* **27**: 421–427.

Moore, A.J. & Pizzari, T. 2005. Quantitative genetic models of sexual conflict based on interacting phenotypes. *Am. Nat.* **165**: S88–S97.

Ng, C.S. & Kopp, A. 2008. Sex combs are important for male mating success in Drosophila melanogaster. *Behav. Genet.* **38**: 195–201.

Parker, G.A. 1979. Sexual selection and sexual conflict. In: *Sexual Selection and Reproductive Competition in Insects* (M. S. Blum & N. A. Blum, eds), pp. 123–166. Academic Press, New York.

Pattatucci, A.M., Otteson, D.C. & Kaufman, T.C. 1991. A functional and structural analysis of the sex combs reduced locus of *Drosophila melanogaster*. *Genetics* **129**: 423–441.

Pfennig, K.S. 1998. The evolution of mate choice and the potential for conflict between species and mate-quality recognition. *Proc. Roy. Soc. Lond. B Biol. Sci.* **265**: 1743–1748.

Pfennig, K.S. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behav. Ecol.* **11**: 220–227.

Pfennig, K.S. & Pfennig, D.W. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. *Q. Rev. Biol.* **84**: 253–276.

Pfenning, K.S. & Hurlbert, A.H. 2012. Heterospecific interactions and the proliferation of sexually dimorphic traits. *Curr. Zool.* **58**: 453–462.

Polak, M. & Simmons, L.W. 2009. Secondary sexual trait size reveals competitive fertilization success in *Drosophila bipectinata* Duda. *Behav. Ecol.* **20**: 753–760.

Polak, M. & Starmer, W.T 2005. Environmental origins of sexually selected variation and a critique of the fluctuating asymmetry-sexual selection hypothesis. *Evolution* **59**: 577–585.

Polak, M. & Tomkins, J.L. 2012. Developmental instability as phenodeviance in a secondary sexual trait increases with fitness-damaging thermal stress. *J. Evol. Biol.* **25**: 277–287.

Polak, M., Starmer, W.T. & Wolf, L.L. 2004. Sexual selection for size and symmetry in a diversifying secondary sexual character in *Drosophila bipectinata* Duda (Diptera: Drosophilidae). *Evolution* **58**: 597–607.

Reuter, M., Linklater, J.R., Lehmann, L., Fowler, K., Chapman, T. & Hurst, G.D.D. 2008. Adaptation to experimental alterations of the operational sex ratio in populations of *Drosophila melanogaster*. *Evolution* **62**: 401–412.

Rice, W.R. & Holland, B. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE) and the intraspecific red queen. *Behav. Ecol. Sociobiol.* **41**: 1–10.

Robertson, F.W. 1962. Changing the relative size of the body parts of Drosophila by selection. *Genet. Res.* **3**: 169–180.

Robinson, S.P., Kennington, W.J. & Simmons, L.W. 2012. Assortative mating for relatedness in a large naturally occurring population of *Drosophila melanogaster*. *J. Evol. Biol.* **25**: 716–725.

Ryan, M.J. & Rand, A.S. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47**: 647–657.

Servedio, M.R. & Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Ann. Rev. Ecol. Evol. Syst.* **34**: 339–364.

Snook, R.R., Robertson, A., Crudgington, H.S. & Ritchie, M.G. 2005. Experimental manipulation of sexual selection and the evolution of courtship song in *Drosophila pseudoobscura*. *Behav. Genet.* **35**: 245–255.

Snook, R.R., Brüstle, L. & Slate, J. 2009. A test and review of the role of effective population size on experimental sexual selection patterns. *Evolution* **63**: 1923–1933.

Snook, R.R., Bacigalupe, L.D. & Moore, A.J. 2010. The quantitative genetics and coevolution of male and female reproductive traits. *Evolution* **64**: 1926–1934.

Spieth, H.T. 1952. Mating behavior within the genus Drosophila (Diptera). *Bull. Am. Mus. Nat. Hist.* **99**: 395–474.

Struhl, G. 1982. Genes controlling segmental specification in the Drosophila thorax. *Proc. Natl. Acad. Sci. USA* **79**: 7380–7384.

Tanaka, K, Barmina, O. & Kopp, A. 2009. Distinct developmental mechanisms underlie the evolutionary diversification of Drosophila sex combs. *Proc. Natl Acad. Sci. USA* **106**: 4764–4769.

Wagner, A. 2011. Genotype networks shed light on evolutionary constraints. *Trends Ecol. Evol.* **26**: 577–584.

Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual selection. *Evolution* **58**: 1028–1037.

Wigby, S., Sirot, L.K., Linklater, J.R., Lehmann, L., Buehner, N., Calboli, F.C. *et al.* 2009. Seminal fluid protein allocation and male reproductive success. *Curr. Biol.* **19**: 751–757.