Evol Ecol
DOI 10.1007/s10682-007-9208-2

ORIGINAL PAPER

Female polymorphisms, sexual conflict and limits to speciation processes in animals

Erik I. Svensson · Jessica K. Abbott · Thomas P. Gosden · Audrey Coreau

Received: 15 March 2007 / Accepted: 23 August 2007

Abstract Heritable and visually detectable polymorphisms, such as trophic polymorphisms, ecotypes, or colour morphs, have become classical model systems among ecological geneticists and evolutionary biologists. The relatively simple genetic basis of many polymorphisms (one or a few loci) makes such species well-suited to study evolutionary processes in natural settings. More recently, polymorphic systems have become popular when studying the early stages of the speciation process and mechanisms facilitating or constraining the evolution of reproductive isolation. Although colour polymorphisms have been studied extensively in the past, we argue that they have been underutilized as model systems of constraints on speciation processes. Colouration traits may function as signalling characters in sexual selection contexts, and the maintenance of colour polymorphisms is often due to frequency-dependent selection. One important issue is why there are so few described cases of female polymorphisms. Here we present a synthetic overview of female sexual polymorphisms, drawing from our previous work on female colour polymorphisms in lizards and damselflies. We argue that female sexual polymorphisms have probably been overlooked in the past, since workers have mainly focused on male-male competition over mates and have not realized the ecological sources of genetic variation in female fitness. Recent experimental evolution studies on fruit flies (*Drosophila melanogaster*) have demonstrated significant heritable variation among female genotypes in the fitness costs of resistance or tolerance to male mating harassment. In addition, female-female competition over resources could also generate genetic variation in female fitness and promote the maintenance of female sexual polymorphisms.

E. I. Svensson (✉) · T. P. Gosden
Section for Animal Ecology, Ecology Building, Lund University, Lund 223 62, Sweden
e-mail: erik.svensson@zooekol.lu.se

J. K. Abbott
Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

A. Coreau
Centre d'Ecologie Fonctionnelle et Evolutive (CEFE-CNRS, UMR 5175), 1919 route de Mende,
Montpellier Cedex 5 34293, France

Female sexual polymorphisms could subsequently either be maintained as intrapopulational polymorphisms or provide the raw material for the formation of new species.

Keywords Antagonistic coevolution · Female competition · Female resistance · Male mating harassment · Sexual conflict · Speciation

Introduction

The ecology of speciation and mechanisms of, and genetic constraints on speciation is a central research focus among evolutionary biologists (Rice 1998; Schluter 1998a; Higashi et al. 1999; Dieckmann and Doebeli 1999; Schluter 2000; Gavrilets 2004; Coyne and Orr 2004). With the exception of a few well-studied, non-traditional model organisms (Schluter 1998b; Higgie et al. 2000; Nosil et al. 2002; Nosil and Crespi 2004), much work in the area of speciation has been focused on classical model organisms such as *Drosophila* (Noor 1995; Noor et al. 2001). Much of the work in this field is primarily theoretical, and recently some prominent evolutionary theoreticians have argued that the imbalance between abundant theory and limited empirical data to test available theory has hampered progress in speciation research (Turelli et al. 2001).

Although research on *Drosophila* and similar model organisms allow high experimental precision, most studies are performed in artificial laboratory conditions, which limit the inferences that can be made about natural populations. On the other hand, research on non-model organisms is hampered by the difficulty of obtaining information about the heritable basis of ecologically important traits that influence the speciation process. Thus, workers interested in the ecology of speciation are caught in a methodological dilemma: either study well-characterized model organisms in artificial laboratory conditions and obtain high genetic precision, or study organisms in their natural environments but sacrifice high genetic precision.

One possible solution to the dilemma of combining ecological studies with easily accessible genetic information in speciation studies in natural populations, is to study heritable “marker phenotypes”, i. e. heritable and conspicuous phenotypic polymorphisms (Smith 1993; Leimar 2005; Svensson and Abbott 2005). Such morphs or ecotypes have their laboratory counterpart in classical *Drosophila* studies in which marker phenotypes such as eye-colour mutants have been used to study genetic dynamics in laboratory evolution assays (Rice 1994). Although polymorphisms in ecologically important traits, such as trophic morphologies or colouration traits have been studied in the past by ecological geneticists (Wright 1969; Dobzhansky 1970; Ford 1975; Eckert and Barrett 1995; Schemske and Bierzychudek 2001), such natural and conspicuous polymorphisms have, until quite recently, been underutilized as model systems of speciation processes (Gray and McKinnon 2007). When such polymorphisms have a known genetic basis (typically one or a few loci) morph-frequency differences between populations and/or generations can be analyzed using the framework of classical population genetics. Temporal and spatial differences in morph frequencies can then potentially be attributed to the action of the well-recognized evolutionary forces of genetic drift, natural and/or sexual selection (Svensson et al. 2005; Svensson and Abbott 2005).

In this article, we will review and discuss the use of sexual polymorphisms in speciation studies. By sexual polymorphisms, we mean the presence of several discrete heritable variants that co-exist within local populations, and which are subject to selection pressures arising from classical sexual selection (mate choice) or sexual conflict. Such heritable morphs could be expressed as visual signalling traits related to sexual reproduction, and then often as colour morphs, although this may not always be the case. In principle, any intraspecific variety that has its main function in sexual reproduction should be classified as a sexual polymorphism in our scheme, and it could be expressed as morphological differences between morphs or olfactory morphs, as long as there is evidence that the traits are true heritable polymorphisms, and not only a phenotypically plastic varieties ("polyphenisms"). Behavioural traits, such as female preference polymorphisms, which are crucial elements in models of sexual selection by female choice (Kirkpatrick 1982), would also be included in our broad definition of sexual polymorphisms. Our definition of sexual polymorphisms does, however, exclude developmentally plastic polyphenisms which have recently been reviewed at length elsewhere (West-Eberhard 2003) as well as purely behavioural and flexible (non-genetic) intraspecific variation, or so-called "alternative reproductive tactics". We will restrict the discussion to heritable animal polymorphisms, partly because of our own taxonomic bias and also because plant evolutionary biologists have a stronger tradition of using such heritable polymorphisms in evolutionary studies (Ågren and Ericson 1996; Barrett 1998; Schemske and Bierzychudek 2001) and such studies would merit a separate review on plant polymorphisms.

Polymorphic animal species, such as discrete colour polymorphisms, were popular study objects during the 1940s and 1950s as part of the strong tradition of "ecological genetics" that flourished in Britain and continental Europe (Ford 1975). Interest in these study systems declined during the later half of the 20th century, presumably because of the development of evolutionary quantitative-genetic theory (Lande 1976) and an increased focus on quantitative phenotypic traits, rather than discrete polymorphisms. With the recent and increasing interest in the early stages of speciation, in particular sympatric speciation and "evolutionary branching" (Dieckmann and Doebeli 1999; Doebeli and Dieckmann 2000) and the recognition that disruptive selection may be more common in nature than was previously thought (Kingsolver et al. 2001) time may now be ripe to return to these classical polymorphisms. The known heritable basis of such polymorphisms avoids the confounding effects of environmental induction and phenotypic plasticity that plagues many studies of quantitative phenotypic traits in natural populations.

Here, our focus will be on sexual polymorphisms, sexual selection and sexual conflict, since trophic polymorphisms have been subject to several recent extensive reviews (Schluter 2000; Robinson and Schluter 2000). We will use examples from our own previous work on colour polymorphism dynamics in lizards and damselflies to discuss the relatively neglected links between female competition, female resistance towards male mating harassment and factors maintaining genetic variation in female fitness. A major conclusion from this paper is that female sexual polymorphisms have probably been overlooked by previous investigators and we would like to encourage more work in this area. There is clearly a need for a synthesis between studies of intrapopulational polymorphisms, such as female sexual polymorphisms, and studies of speciation processes in natural populations (Gray and McKinnon 2007).

Genetic assumptions in studies of sexual polymorphisms: ESS and adaptive dynamics

Sexual polymorphisms in animals have previously mainly been described in males as alternative reproductive strategies, in the framework of game theory and "Evolutionarily Stable Strategies" (ESS) (Maynard Smith 1982, 1996). In these models, it is usually assumed that there are two or more alternative reproductive strategies with equal mating success (or fitness). Strategies are assumed to be heritable, and inheritance is assumed to be asexual. Similar simplifying assumptions about a basic asexual inheritance are also made in the closely related modelling approach of “adaptive dynamics”(Dieckmann and Doebeli 1999, 2005). Valid criticisms from population geneticists and speciation theorists against these highly questionable assumptions of asexual inheritance in game theory and adaptive dynamics (Waxman and Gavrilets 2005a, b) are unfortunately too often dismissed or played down by the enthusiastic supporters of these phenotypic modelling approaches (Kokko 2001, 2005 Kokko et al. 2002). Our own view is that the study of speciation cannot have as a basic assumption that inheritance is asexual, given the importance of recombination as a constraint on speciation, particularly sympatric speciation (Felsenstein 1981). Thus, we largely agree with Waxman and Gavrilets that, although game theory and adaptive dynamics may be fruitful modelling approaches in ecology, their underlying assumptions about asexual inheritance makes the utility of these approaches questionable in the study of speciation processes (Waxman and Gavrilets 2005a, b).

The empirical study of sexual polymorphisms would benefit considerably from a broadening of the classical ESS-approach to also include details about genetic architecture, such as the number of loci and dominance relationships between alleles. Neither classical game theory nor adaptive dynamics are suitable modelling approaches to deal with these issues, in contrast to classical population genetics. In particular, the role of allelic dominance in speciation processes and sexual polymorphisms is likely to be both ecologically and evolutionarily important (Van Dooren 1999), and we will return to this issue later.

The ecological causes and origin of male sexual polymorphisms

The evolutionary origins of male sexual polymorphisms are usually caused by male-male competition over mates (Shuster and Wade 2003). Such intense male-male competition over access to females stimulated early workers to suggest that variance in male fitness is usually higher than variance in female fitness, and that this pattern arises through differences in the number of obtained mates (Trivers 1972; Shuster and Wade 2003). In contrast, variation in female fitness was usually assumed to be low, at least much lower than in males (Bateman 1948). Such a neglect of variation in female fitness was partly understandable, since it was difficult to understand at that time how such variation could be generated, given the lack of knowledge about costs of mating and sexual conflict.

When male-male competition becomes so severe that one phenotypic class of males is excluded almost entirely from mating, frequency-dependent selection is thought to favour the development of alternative male mating strategies, such as “sneakers”, “female mimics” or less aggressive (satellite) males (Lank et al. 1995; Sinervo and Lively 1996; Shuster and Sassaman 1997). Such genetic polymorphisms in males, driven by frequency-dependent mate-competition, could be expressed as size differences between males (Gross 1985; Shuster and Sassaman 1997) or as differences in signalling traits (e. g. colour) and/or behaviour (Lank et al. 1995; Sinervo and Lively 1996). Because such male sexual polymorphisms have recently been subject to a recent excellent in-depth review (Shuster and Wade 2003) we will not dwell further into the fascinating details of these polymorphisms but will from now on focus on the more neglected female sexual polymorphisms.

Causes of genetic variation in female fitness

Whereas most workers are aware of how variation in male fitness arises (male-male competition), our understanding of how variation in female fitness is generated is more recent. This is the major reason, in our opinion, why female-female competition and female sexual polymorphisms have been often neglected until quite recently. It was difficult for many workers in the past to see how sufficiently large variation in female fitness could be generated to drive strong female-female competition. But advances over the last 10 years in both theory and empirical data have provided two such mechanisms: (1) Genetic and phenotypic variation in "condition" or resource acquisition ability (Houle 1991) and (2) variation in female resistance or tolerance to matings (Linder and Rice 2005; Lew et al. 2006; Gosden and Svensson 2007). Here we define resistance as the ability of females to avoid unwanted matings, and tolerance is the ability of females to minimize the harmful effects (fitness costs) of unwanted matings (Gosden and Svensson 2007). Our definition of the consequences of male–female mating interactions is exactly analogous to the definitions for variation in tolerance and resistance in the interactions between plants and their herbivores (Strauss and Agrawal 1999).

It is often assumed that there is substantial variation in resource availability and condition among genotypes within populations (Rowe and Houle 1996). Standing genetic variation in condition reflects the overall genomic vigour across all loci, and overall condition is the target of high (genome-wide) mutational input (Rowe and Houle 1996). Although this "genic capture"-model of condition has traditionally been discussed in the context of condition-dependent secondary sexual traits in males, the model also applies to female condition and life-history traits (Houle 1992; Houle et al. 1994).

The second source of how variation in female fitness is generated, via female resistance or female tolerance, is more recent. The first insights came from experimental evolution studies of sexually antagonistic co-evolution between males and females (Rice 1996). In an elegant, long-term laboratory evolution experiment, when *Drosophila melanogaster* males were allowed to evolve freely and females were prevented from coevolving with males, males quickly evolved to become more harmful to females as a correlated effect of male-male mate competition (Rice 1996). Evidence from this classical experiment was indirect, since females were inferred to have lost their ability to resist or tolerate male matings when prevented from coevolving with these males. Later studies using the same system have quantified the significant genetic variation among female genotypes in their resistance and tolerance to males (Linder and Rice 2005; Lew et al. 2006). A major part of the standing genetic variation in female resistance to harm from males appears to be variation among female genotypes in the tendency to remate (Linder and Rice 2005). Although the estimated heritability of female resistance was rather low in these studies (2–3%) they clearly indicate that different female genotypes show differential susceptibility to male mating harassment, and thus demonstrate an important mechanism of how genetic variation in female fitness can be generated. The relative importance of female resistance and tolerance to matings and the link to both total female fitness and fitness components remains to be investigated in natural populations (Gosden and Svensson 2007).

Female sexual polymorphisms in natural populations

Whereas there are many examples of alternative mating strategies and heritable polymorphisms in males, there are still very few such polymorphisms that have been described among females. However, we should expect to find such polymorphisms, given the presence of additive genetic variation for female resistance to male mating attempts (see above) and molecular evidence for allelic polymorphisms at reproductive loci in both males and females (Palumbi 1999). It is important to underscore that sexual polymorphisms may not always be possible to detect visually, but may be cryptic and only detectable by molecular analyses of the underlying reproductive loci. For instance, studies of the molecular basis of gamete recognition loci in the sea urchin genus *Echinometra* have revealed extensive polymorphisms in traits important to fertilization success among male sperm haplotypes (Palumbi 1999). It seems likely that there may be additional hidden phenotypic differences among female genotypes in their reproductive performance.

Visually detectable polymorphisms, such as colour polymorphisms, may have gone unnoticed in females by previous investigators. One example of this is the recent description of a female throat colour polymorphism in the common lizard *Lacerta vivipara* (Vercken et al. 2007) which is found throughout Eurasia. This species has been studied extensively in the past, without any previous scientific documentation of this polymorphism. Vercken and colleagues found three female ventral colour morphs in this species: pale yellow, orange and intermediate (Vercken et al. 2007). These female morphs are stable through an individual's lifetime and are maternally heritable. The three female morphs also respond differently in terms of fitness parameters (clutch size, hatching success and litter sex ratio) to changing density- and frequency-dependent social environments (Vercken et al. 2007). This newly discovered female polymorphism resembles the previously described female throat colour polymorphism in an unrelated lizard relative from another family on another continent: the side-blotched lizard *Uta stansburiana*, in North America (Sinervo et al. 2000). In side-blotched lizards, there is evidence for strong and fluctuating selection pressures on female reproductive traits (clutch size and egg mass), and these selection pressures are driven by density- and frequency-dependent social interactions among both hatchlings and adult females (Sinervo et al. 2000). The two heritable female throat colour morphs in this species (orange and yellow) differ in their sensitivity to these changing social conditions and throat colour is genetically correlated with several fitness-related traits such as clutch size, egg mass (Sinervo et al. 2000), and immune function (Svensson et al. 2001).

These two lizard species are perhaps the two most in-depth studies available of female sexual polymorphisms to date. Apart from these quantitative studies, female colour polymorphisms have also been studied in damselflies, where two or three heritable colour morphs exist in several different genera, such as *Coenagrion*, *Enallagma* and *Ischnura* (Cordero 1990; Cordero et al. 1998; Andrés et al. 2000; Svensson et al. 2005). In these damselfly species, males are usually monomorphic. Similar female-limited polymorphisms occur in several species of butterflies, such as those in the genus *Colias* (Nielsen and Watt 2000; Ellers and Boggs 2003) and in hummingbirds, where male-coloured females occur in several different species (Bleiweiss 2001). We will address the ecological causes of the maintenance of these female colour polymorphisms in next section, but we note that in both the damselflies and butterflies, an important role for male mating behaviour and/or male mating harassment have been either shown or implicated (Ellers and Boggs 2003; Fincke 2004; Svensson et al. 2005).

Female colour polymorphisms have also been reported in fish (McKinnon et al. 2000; Pierotti and Seehausen 2007) and several species of birds (Bleiweiss 2001; Roulin et al. 2003; Brommer et al. 2005). In three-spine sticklebacks *Gastrosteus aculeatus*, the intensity of orange-red throat colouration appears to be non-randomly associated with migratory behaviour, since there are differences between stream-resident and anadromous females, whereas no such differences were found between males (McKinnon et al. 2000). Similarly, in the African cichlid species *Neochromis omnicaeruleus*, female colour morphs appears to be a target of male mate choice and presumably play an important role in speciation processes in this cichlid species flock (Lande et al. 2001; Pierotti and Seehausen 2007). These fish studies strongly suggest that female colour variation and female polymorphisms are not simply a correlated response to sexual selection in males. Thus, female colour morphs are likely to be targets of direct selection with male mating behaviour playing key role, as is the situation in butterflies and damselflies. In tawny owls *Strix aluco*, there is a colour polymorphism that is present in both males and females: reddish-brown and grey phenotypes (Roulin et al. 2003; Brommer et al. 2005). The ecological and physiological consequences of these colour differences are uncertain but they may be related to temperature tolerance. These female colour morphs differ in size, annual survival and reproductive traits (Roulin et al. 2003; Brommer et al. 2005).

Sexually antagonistic selection and female polymorphisms

Most studies of polymorphic animal species have, until quite recently, focused on male polymorphisms and in only a few cases are the intrapopulational selection pressures that maintain these polymorphisms well known or described (Gray and McKinnon 2007). When negative frequency-dependent selection (NFDS) maintains multiple female morphs within populations, one relatively neglected selective force could be frequency-dependent sexual conflict over mating rates (Gavrilets and Waxman 2002; Svensson et al. 2005). In such antagonistic male–female mating interactions, males are thought to “chase” females over evolutionary time, while females can respond by evolving various kinds of resistance to unmated male mating attempts (Arnqvist and Rowe 2002; Rowe and Arnqvist 2002; Linder and Rice 2005; Lew et al. 2006). The outcome of this coevolutionary chase could either be the divergence of populations and speciation (either in sympatry or allopatry) or the formation of stable genetic clusters, i. e. female polymorphisms, within populations (Gavrilets and Waxman 2002). Although this latter idea has received theoretical interest, there is very little evidence in natural populations for such stable female genetic clusters (or morphs) that arise from and are maintained by sexual conflict.

One promising study system is provided by several species of diving beetles (Dytiscidae). In these beetles, males have numerous suction disks varying in size and numbers which help the males attach themselves to the female’s back during mating (Hardling and Bergsten 2006). Some females have modified backs (compared to males) with furrows, hairs, punctures, or granulations of the dorsum. These modified structures make it harder for males without suitable suction disks to attach to females carrying these structures, compared to normal smooth (male-like) females (Hardling and Bergsten 2006). Variation in female dorsum structure thus appears to be a case of an intrasexual polymorphism that has evolved to enable females to escape from unwanted male mating attempts (Hardling and Bergsten 2006). This female polymorphism is presumably maintained by NFDS in which a female morph that reaches a sufficiently high frequency suffers from excessive male mating harassment, causing its fitness and morph frequency in the next generation to decline (Hardling and Bergsten 2006).

Another example of an intrasexual polymorphism that is presumably maintained by antagonistic male–female mating interactions is provided by the blue-tailed damselfly (*Ischnura elegans*) (Fig. 1). Over the past 7 years, we have performed in-depth studies of this species in a series of populations in southern Sweden (Svensson et al. 2005; Abbott and Svensson 2005; Svensson and Abbott 2005). Females in *I. elegans* occur in a number of colour varieties, of which some are age-related and three are mature phases that are the adult female colour morphs (Fig. 1). Males are monomorphic with respect to colour, and hence this polymorphism is sex-limited in its expression. Laboratory breeding experiments in both *I. elegans* and hybridization data with its close relative *I. graellsii* have shown that female colour morph is inherited as one autosomal locus with three alleles in a dominance hierarchy (Cordero 1990; Sanchez-Guillen et al. 2005). The most common female morph in our populations is male-like in both coloration and melanin patterning, and females with this phenotype are called "androchrome" (male-coloured) females (Fig. 1). The two other female morphs, *infuscans* and *infuscans-obsoleta*, have different colouration and/or melanin patterning than males (Fig. 1). Note that the dominance relationships of the three alleles means that females carrying only one androchrome allele (here denoted p) will express the androchrome phenotype, since p is dominant over both the *infuscans*-allele (q) and the *infuscans-obsoleta*-allele (r).

It has been suggested that androchrome females may be a form of "male mimic", given their similar visual appearance to males (Cordero et al. 1998). The adaptive advantage of such male mimicry would be that androchrome females could lower their detection rate by males, thereby avoiding costly male mating harassment and hence reduce their mating rates (Cordero et al. 1998). In line with this, androchrome females are

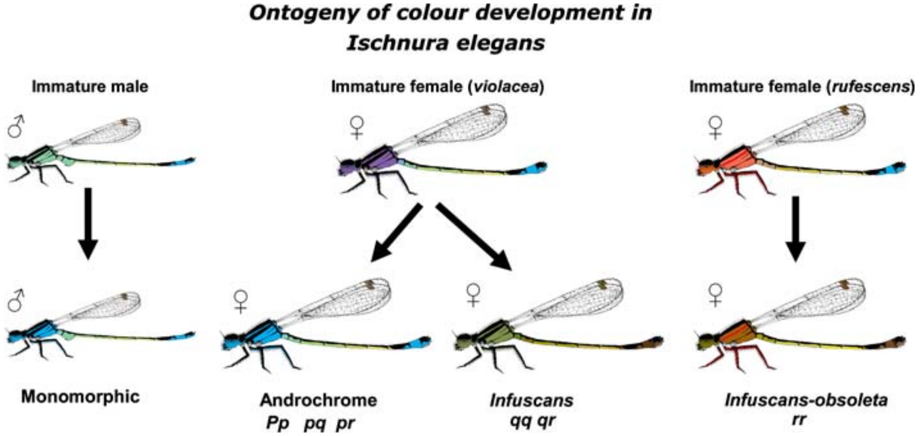

Fig. 1 Ontogeny of colour development in the three heritable morphs in *Ischnura elegans.* Note that there are both age- and morph-related colour differences, and that the males in this species are monomorphic. Males are green in the immature stage and become blue when sexually mature. One of the immature colour phases (*violacea*) subsequently matures in to either an adult blue morph ("male mimic"; androchrome females) or a more olive-green morph (*infuscans*). Androchrome females have both male-like blue colouration and melanin patterning (three dark bands on thorax), whereas *infuscans*-females only have male-like melanin patterning. The juvenile colour phase rufescens subsequently matures to the adult morph *infuscans-obsoleta* which differs from males in both colouration and melanin patterning (*infuscans-obsoleta* females only have one dark melanin band on their thorax). Note that both the *violacea* and *rufescens* colour phases have male-like colouration on their 8th abdominal segments (violet and blue, respectively), whereas this colour disappears at maturity in infuscans and infuscans-obsoleta females, which have brown 8th segments. Since neither of these two immature phases are often found mating with males in the field, and the adult androchrome females also have lower mating rates, the colour on the 8th segment may function as a mate-avoidance signal, caused by male similarity. Below the adult morphs we have written the six possible genotypes that underlie these three adult visible morphs using standard Hardy-Weinberg abbreviation of the three alleles involved in morph determination and their dominance order: p (androchrome) > q (*infuscans*) > r (*infuscans-obsoleta*). Illustration of morphs by J. K. Abbott

also more male-like in their body shape, and we have recently found that the male-like body shape of androchromes persists when families of *I. elegans* are raised in a common laboratory environment, indicating that these shape differences between morphs are heritable (Abbott and Svensson 2007). An alternative, although not mutually exclusive, explanation for the maintenance of multiple female morphs in *I. elegans* and other damselfly species is that males form a "search image" for the most common morph at any one time in a local population. Common morphs thereafter suffer from more intensive male-mating harassment, causing both their fitness and future frequency to decline (Fincke 2004). This latter mechanism is thus a form of apostatic selection, similar to the frequency-dependent visual mechanism that causes the fitness of common prey to decline, due to the development among predators of search images towards common prey morphs (Bond and Kamil 1998, 2002). Evidence for apostatic selection in terms of frequency-dependent female fecundities comes from our longitudinal study of four generations, involving a dozen populations (Svensson et al. 2005; Svensson and Abbott 2005). Female relative fecundities were clearly frequency-dependent, and decreased when a morph became more common, exactly consistent with the expectation from the apostatic selection hypothesis (Svensson et al. 2005). Moreover, female morph frequencies changed rapidly and significantly between generations, and these rapid evolutionary dynamics in the genetic composition of the populations appears to be non-random and not solely explained by the stochastic effects of genetic drift (Svensson et al. 2005; Svensson and Abbott 2005). Finally, the proportion of androchrome females found *in copula*, which can be used as a proxy for mating rate, was both frequency- and density-dependent, since androchrome females were found more often in copula when male density was high and when androchrome frequency was high (Svensson et al. 2005). Thus, several independent lines of evidence strongly suggest that density- and frequency-dependent sexual conflict maintains this female polymorphism, although the link to male mating harassment needs to be experimentally verified. Here we present additional field evidence in support of male mating harassment being the causal factor driving female morph frequency-dynamics in this system.

We have carried out field experiments, in which individuals of the two most common female morphs (androchrome and *infuscans*-females) were tethered to a common perch, and counted the number of male visits to each morph. In these field experiments, we found that *infuscans*-females were approached by almost three times more *I. elegans* males per unit time than were androchrome females (Fig. 2A). The higher male visitation rate of *infuscans*-females in these experiments implies that this morph is more easily detectable or attractive to males and/or more often perceived as being a female, compared to androchromes. This will of course also depend on the actual morph frequencies in the population. The *infuscans*-females have 15–21% higher fecundity than the two other morphs (Svensson and Abbott 2005), which could contribute to make them more attractive to males. Indeed, adaptive male mate choice of more fecund females is increasingly being recognized as important in insects (Bonduriansky 2001; Byrne and Rice 2006). However, it also seems likely that the net male fitness benefits of searching for more fecund *infuscans*-females will be frequency-dependent and will decline if this morph becomes sufficiently rare in a given population. Male search costs for *infuscans*-females will then increase, making it more profitable to switch target female morph. Males could then be expected to switch their search efforts to more male-like phenotypes, i. e. androchromes. This requires that damselfly males have phenotypically plastic search images and/or learned mate preferences, for which there is now experimental evidence (Van Gossum et al. 2001; Fincke et al. 2007).

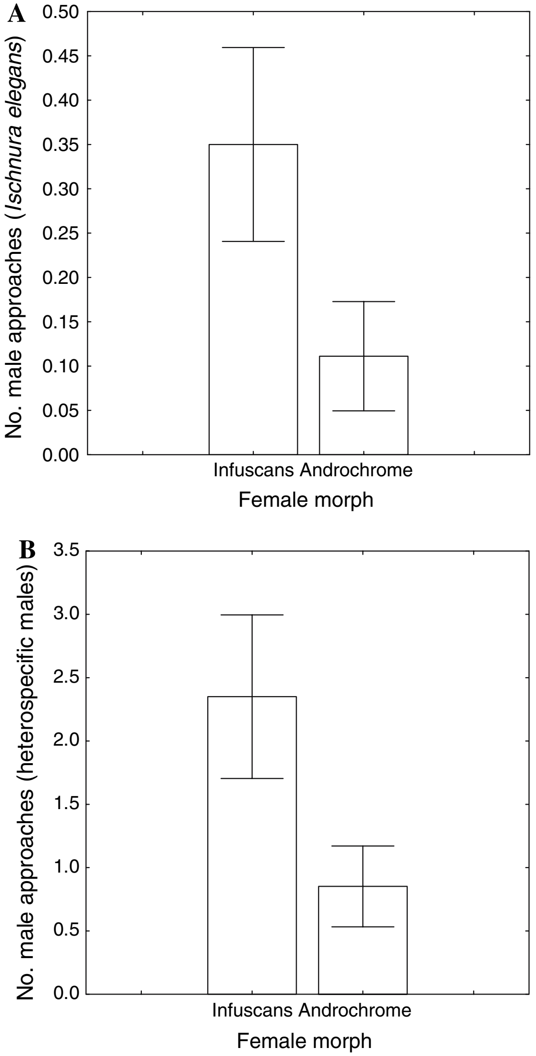

Fig. 2 Males approach females of the *infuscans*-morph more often in the field, compared to the male-mimicking androchrome morph, indicating higher male mating harassment of *infuscans* females. Data come from tethering experiments in the field during late July and early August 2002. *Infuscans*- and androchrome females were tied with a fishing line to a bamboo stick in the field, and the number of approaching males was counted during 15 min intervals. (**A**) Males of *I. elegans* approach *infuscans*-females at a three times higher rate than the approach rate for androchrome females: $F_{1,45} = 4.088$; $P = 0.049$. (**B**) Heterospecific males, mainly *Enallagma cyathigerum* and *Coenagrion pulchellum*, also approach *infuscans*-females at a higher rate than androchrome females: $F_{1,45} = 5.046$; $P = 0.030$. These experiments show that males of both *I. elegans* and of other species are clearly able to discriminate between these two female colour morphs. *Infuscans-obsoleta* females were not included in these experiments due to low frequencies and associated low sample sizes in the field

Interestingly, the number of approaches by heterospecific males from other damselfly species (mainly *Coenagrion pulchellum* and *Enallagma cyathigerum*) was also elevated for *infuscans*-females, relative to androchrome females (Fig. 2B). Thus, there may also be a role for heterospecific male mating harassment in the dynamics of this polymorphism, in addition to harassment from conspecific males (Johnson 1975). The reason for the biased approaches by heterospecific males may be because females in *Enallagma cyathigerum* are mainly brownish gynochromes (female-coloured), and androchrome frequencies in this species are low, around 10% in our study populations (T. P. Gosden and E. I. Svensson, unpublished data), while androchrome frequencies in *I. elegans* range from about 60–80% in our study populations (Svensson and Abbott 2005). Thus, heterospecific males which are sympatric with *I. elegans* may have a search image that primarily targets brownish females, and may therefore perceive *infuscans*-females of *I. elegans* (but not androchrome

I. elegans females) as resembling females of their own species. Such a role for mistaken species identity and heterospecific male mating harassment, in addition to intraspecific male mating harassment, has also been suggested and shown to operate in the female colour polymorphism involving male-like morphs in *Colias* butterflies in North America (Nielsen and Watt 2000). Thus, variation in the strength of interspecific interactions mediated by local species composition could potentially play a role in explaining population variation in morph frequencies (Johnson 1975). More generally, polymorphic species have been underutilized in the study of character displacement and interspecific competition, although such taxa may provide some of the most powerful systems to test these classical competition theories in community ecology (Rice and Pfennig 2007).

Finally, we have recently initiated new field experiments to directly estimate the fecundity-consequences of mating rates in the different female morphs in both *I. elegans* and *E. cyathigerum*. We have applied fluorescent dust of different colours to the male claspers (cerci) and genitalia in the field, modifying a previous experimental protocol used in studies of butterfly inbreeding depression (Joron and Brakefield 2003). Males dusted with different colours have been released in the field, and we have subsequently returned to catch as many females as possible at the same sites, thereby enabling us to estimate mating rates of females of different morphs (Fig. 3). Females with different numbers of matings have been set up in ovipositoria to estimate the fecundity consequences of differing mating rates. Results show that the female morphs in *I. elegans* differ in their resistance to male mating attempts, the average number of matings and the effect multiple matings have on fecundity, i.e. female tolerance to male mating attempts and realized matings (Gosden and Svensson 2007).

Stably maintained polymorphism or speciation?

Some intrasexual polymorphisms, such as the diving beetles and damselflies that have been described above, are clearly consistent with the idea that frequency-dependent sexual selection could promote and maintain polymorphisms. The existence of such female sexual polymorphisms, which continue to coexist sympatrically in local populations, reveals an alternative outcome of sexual conflict to the more traditionally invoked scenario of ever-escalating arms races and speciation (Rice 1996, 1998; Rice and Holland 1997; Gavrilets 2000; Arnqvist et al. 2000). This alternative outcome of sexual conflict, i. e. a stably maintained genetic polymorphism in females rather than the evolution of reproductive isolation, has emerged in recent models of sympatric speciation by sexual conflict (Gavrilets and Waxman 2002; Gavrilets 2004). Sexual conflict as a constraint, rather than as a promoter of population divergence and speciation, has also been suggested in at least one other theoretical model (Parker and Partridge 1998) and in some empirical studies (Magurran 1998).

Other important factors that could influence the outcome of sexual conflict (polymorphism or subsequent speciation) is the genetic architecture of the female sexual traits. The genetic architecture includes the number of loci, their specific effects and dominance relationships between alleles (Hayashi et al. 2007) and the strength of assortative mating and the resulting linkage disequilibria between male and female trait alleles (Hardling and Bergsten 2006). When the traits subject to sexual conflict have relatively simple genetic architectures and there is dominance between alternative alleles, such as in the one-locus colour polymorphism in *Ischnura*-damselflies, the continued segregation of a female sexual polymorphism could be a more likely outcome than speciation. Frequency-

Fig. 3 Colour dusting experiments in *Enallagma cyathigerum*. The main picture shows a newly dusted male *E.cyathigerum*. Note the powder on the genitals (at the join of the thorax and abdomen) and claspers (end of the abdomen). As males clasp females before mating, the dust on the male clasping organs (cerci) is transferred to the female thorax with a male mating attempt. If the female responds by bring her abdomen up to form what is known as the tandem wheel position then dust will be transferred to the female's genitals. This made it possible to check females for the presents of dust both on the thorax (inset bottom left) and female genitals on the end of the abdomen (inset bottom right) using a dissecting microscope and UV light (inset top right). Dust spots of different colours then provides a measure of how many times the female was grabbed by males dusted with different colours, and how many times she responded and mated (minimum estimate, depending on the number of dust colours used). The dust is present on the male for the rest of his life-time, as is shown on a male caught two weeks after dusting (inset top left)

dependent disruptive selection may also, in itself, promote a simple genetic architecture by favouring traits that will increasingly become governed by only one or a few genes of major effect, even if the ancestral condition is a polygenic trait (Kopp and Hermisson 2006). Selection may also favour the evolution of dominance among alleles, making heterozygotes more similar to one of the homozygotes, to ameliorate heterozygote disadvantage (Van Dooren 1999; Rueffler et al. 2006). In this last scenario, there will be a "race" between how fast the evolution of dominance evolves, compared to how fast the evolution of reproductive isolation between sympatric morphs evolves, and if dominance "wins" a stable polymorphism will be the result, rather than sympatric speciation (T. J. M. Van Dooren, pers. comm.). Similar arguments could apply to the evolution of sex-limited gene expression, and we note that the polymorphism in *I. elegans* is characterized by both sex-limited expression to females and allelic dominance (Fig. 1).

Available phylogenetic evidence does not allow any firm conclusion as to which one of the three female morphs arose first in *I. elegans* (Fincke et al. 2005). This is not surprising, given the difficulty and low statistical power when estimating ancestral character states for rapidly evolving characters (Schluter et al. 1997). The evolution of sex-limited gene expression of traits is thought to result either from linkage between sex-determining genes and sexual traits or due to the spread of sex-limited autosomal mutations (Rhen 2000). In the case of *I. elegans*, the latter possibility is more plausible, given the autosomal inheritance of colour morph in this species (Sanchez-Guillen et al. 2005). However, we note that sex-limited expression may be restricted to colour and it is certainly not complete in *I. elegans*, since sons from the different morphs differ in their development time and body shape (Abbott and Svensson 2005, 2007). We have suggested that such incomplete sex-limitation could potentially fuel an intersexual ontogenetic sexual conflict (Rice and Chippindale 2001) between alleles for male protandry and the morph-locus which affects development time in both males and females (Abbott and Svensson 2005).

Conclusions and suggestions for future research

The study of female sexual polymorphisms and the ecological causes of their evolutionary maintenance have begun only in the last few years. We suggest that the relative neglect of female polymorphisms in natural populations may be due to a lack of knowledge of the powerful force of frequency-dependent sexual conflict and female fitness costs of matings and mating harassment. With a rich body of recently developed theoretical models in combination with data from laboratory selection experiments on model organisms like *Drosophila melanogaster*, many workers should now be well aware of the potential importance of sexual conflict over mating. The theoretical models have so far almost exclusively been tested and evaluated in laboratory settings, and crucial elements of these models remain to be investigated in natural populations. We encourage future workers to choose systems with heritable and conspicuous female sexual polymorphisms as model systems and investigate the potential role of frequency-dependent sexual conflict in maintaining such polymorphisms.

Acknowledgements This study was financially supported by grants from the Swedish Research Council (VR), and The Swedish Council for Environment, Agriculture and Spatial Planning (FORMAS) to E. I. S. We are grateful to Fabrice Eroukhmanoff, Roger Härdling, Kristina Karlsson, Anna Runemark and other members of our research laboratory for stimulating discussions on female polymorphisms over the past years, and to three anonymous referees for criticisms on the first version of this manuscript. We also wish to thank Ola Fincke for providing us with a pre-print of her recent paper that was in press when we finished this article.

References

Abbott J, Svensson EI (2005) Phenotypic and genetic variation in emergence and development time of a trimorphic damselfly. J Evol Biol 18:1464–1470

Abbott JK, Svensson EI (2007) Ontogeny of sexual dimorphism and phenotypic integration in heritable morphs. Evol Ecol doi:10.1007/s10682-007-9161-0

Ågren J, Ericson L (1996) Population structure and morph-specific fitness differences in tristylous *Lythrum salicaria*. Evolution 50:126–139

Andrés JA, Sánchez-Guillen RA, Cordero Rivera A (2000) Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. Evolution 54:2156–2161

Arnqvist G, Edwardsson M, Friberg U, Nilsson T (2000) Sexual conflict promotes speciation in insects. Proc Natl Acad Sci USA 97:10460–10464

Arnqvist G, Rowe L (2002) Antagonistic coevolution between the sexes in a group of insects. Nature 415:787–789

Barrett SCH (1998) The reproductive biology and genetics of island plants. In: Grant PR (ed) Evolution on islands. Oxford University Press, Oxford, pp 18–34

Bateman AJ (1948) Intra-Sexual selection in *Drosophila*. Heredity 2:349–368

Bleiweiss R (2001) Asymmetrical expression of transsexual phenotypes in hummingbirds. Proc R Soc Lond B 268:639–646

Bond AB, Kamil AC (1998) Apostatic selection by blue jays produces balanced polymorphism in virtual prey. Nature 395:594–596

Bond AB, Kamil AC (2002) Visual predators select for crypticity and polymorphism in virtual prey. Nature 415:609–613

Bonduriansky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305–339

Brommer JE, Ahola K, Karstinen T (2005) The colour of fitness: plumage coloration and lifetime reproductive success in the tawny owl. Proc R Soc B-Biol Sci 272:935–940

Byrne PG, Rice WR (2006) Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proc R Soc B-Biol Sci 273:917–922

Cordero A (1990) The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur)(Odonata:Coenagrionidae). Heredity 64:341–346

Cordero A, Carbone SS, Utzeri C (1998) Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans*. Anim Behav 55:185–187

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates Inc., Sunderland, Massachusetts

Dieckmann U, Doebeli M (1999) On the origin of species by sympatric speciation. Nature 400:354–357

Dieckmann U, Doebeli M (2005) Pluralism in evolutionary theory. J Evol Biol 18:1209–1213

Dobzhansky T (1970) Genetics of the evolutionary process. Columbia University Press, New york

Doebeli M, Dieckmann U (2000) Evolutionary branching and sympatric speciation caused by different types of ecological interactions. Am Nat 156:S45–S61

Eckert CG, Barrett CH (1995) Style morph ratios in tristylous *Decodon verticillatus* (Lythraceae): selection vs. historical contingency. Ecology 76:1051–1066

Ellers J, Boggs CL (2003) The evolution of wing color: male mate choice opposes adaptive wing color divergence in Colias butterflies. Evolution 57:1100–1106

Felsenstein J (1981) Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124–138

Fincke OM (2004) Polymorphic signals of harassed female odonates and the males that learn them support a novel frequency-dependent model. Anim Behav 67:833–845

Fincke OM, Jödicke R, Paulson D, Schultz DT (2005) The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like morphs typically the minority? Int J Odonatol 8:183–212

Fincke OM, Fargevieille A, Schultz TD (2007) Lack of innate preference for morph and species identity in mate-searching *Enallagma* damselflies. Behav Ecol Sociobiol 61:1121–1131

Ford EB (1975) Ecological genetics 4th edn. Chapman and Hall, London

Gavrilets S (2000) Rapid evolution of reproductive barriers by sexual conflict. Nature 403:886–889

Gavrilets S (2004) Fitness landscapes and the origin of species. Princeton University Press, NJ, Princeton

Gavrilets S, Waxman D (2002) Sympatric speciation by sexual conflict. Proc Natl Acad Sci USA 99:10533–10538

Gosden TP, Svensson EI (2007) Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. PLoS ONE 2(6):e580 doi:10.1371/journal.pone.0000580

Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. Trends Ecol Evol 22:71–79

Gross MR (1985) Disruptive selection for alternative life histories in Salmon. Nature 313:47–48

Hardling R, Bergsten J (2006) Nonrandom mating preserves intrasexual polymorphism and stops population differentiation in sexual conflict. Am Nat 167:401–409

Hayashi TI, Vose M, Gavrilets S (2007) Genetic differentiation by sexual conflict. Evolution 61:516–529

Higashi M, Takimoto G, Yanamura N (1999) Sympatric speciation by sexual selection. Nature 402:523–526

Higgie M, Chenoweth S, Blows MW (2000) Natural selection and the reinforcement of mate recognition. Science 290:519–521

Houle D (1991) Genetic covariance of fitness correlates: what genetic correlations are matters. Evolution 45:630–648

Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics 130:195–204

Houle D, Hughes KA, Hoffmaster DK, Ihara J, Assimacopoulus S, Canada D, Charlesworth B (1994) The effects of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. Genetics 138:773–785

Johnson C (1975) Polymorphism and natural selection in Ischnuran damselflies. Evol Theory 1:81–90

Joron M, Brakefield PM (2003) Captivity masks inbreeding effects on male mating success in butterflies. Nature 424:191–194

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P (2001) The strength of phenotypic selection in natural populations. Am Nat 157:245–261

Kirkpatrick M (1982) Sexual selection and the evolution of female choice. Evolution 36:1–12

Kokko H (2001) Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecol Lett 4:322–326

Kokko H (2005) Useful ways of being wrong. J Evol Biol 18:1155–1157

Kokko H, Brooks R, McNamara JM, Houston AI (2002) The sexual selection continuum. Proc R Soc Lond B 269:1331–1340

Kopp M, Hermisson J (2006) The evolution of genetic architecture under frequency-dependent disruptive selection. Evolution 60:1537–1550

Lande R (1976) Natural selection and random genetic drift in phenotypic evolution. Evolution 30:314–334

Lande R, Seehausen O, van Alphen JM (2001) Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. Genetica 112–113:435–443

Lank DB, Smith CM, Hanotte O, Burke T, Cooke F (1995) Genetic polymorphism for alternative mating-behavior in Lekking male Ruff Philomachus-Pugnax. Nature 378:59–62

Leimar O (2005) The evolution of phenotypic polymorphism: randomized strategies versus evolutionary branching. Am Nat 165:669–681

Lew TA, Morrow EH, Rice WR (2006) Standing genetic variance for female resistance to harm from males and its relationship to intralocus sexual conflict. Evolution 60:97–105

Linder JE, Rice WR (2005) Natural selection and genetic variation for female resistance to harm from males. J Evol Biol 18:568–575

Magurran AE (1998) Population differentiation without speciation. Phil Trans R Soc Lond B 353:275–286

Maynard Smith J (1982) Evolution and the theory of games. Cambridge University Press, Cambridge

Maynard Smith J (1996) The games lizards play. Nature 380:198–199

McKinnon JS, Demayo RF, Granquist R, Weggel L (2000) Female red throat coloration in two populations of threespine stickleback. Behaviour 137:947–963

Nielsen MG, Watt WB (2000) Interference competition and sexual selection promote polymorphism in *Colias* (Lepidoptera, Pieridae). Funct Ecol 14:718–730

Noor MA (1995) Speciation driven by natural selection in *Drosophila*. Nature 375:674–675

Noor MAF, Grams KL, Bertucci LA, Reiland J (2001) Chromosomal inversions and the reproductive isolation of species. Proc Natl Acad Sci USA 98:12084–12088

Nosil P, Crespi BJ (2004) Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in Timema cristinae walking-sticks. Evolution 58:102–112

Nosil P, Crespi BJ, Sandoval CP (2002) Host-plant adaptation drives the parallel evolution of reproductive isolation. Nature 417:440–443

Palumbi SR (1999) All males are not created equal: fertility differences depend on game recognition polymorphisms in sea urchins. Proc Natl Acad Sci USA 96:12632–12637

Parker GA, Partridge L (1998) Sexual conflict and speciation. Phil Trans R Soc Lond B 353:261–274

Pierotti MER, Seehausen O (2007) Male mating preferences pre-date the origin of a female trait polymorphism in an incipient species complex of Lake Victoria cichlids. J Evol Biol 20:240–248

Rhen T (2000) Sex-limited mutations and the evolution of sexual dimorphism. Evolution 54:37–43

Rice WR (1994) Degeneration of a nonrecombining chromosome. Science 263:230–232

Rice WR (1996) Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 381:232–234

Rice WR (1998) Intergenomic conflict, interlocus antagonistic coevolution and the evolution of reproductive isolation. In: Howard DJ, Berlocher SH (eds) Endless forms: species and speciation. Oxford University Press, Oxford

Rice WR, Holland B (1997) The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. Behav Ecol Sociobiol 41:1–10

Rice WR, Chippindale AK (2001) Intersexual ontogenetic conflict. J Evol Biol 14:685–693

Rice AM, Pfennig DW (2007) Character displacement: in situ evolution of novel phenotypes or sorting of pre-existing variation? J Evol Biol 20:448–459

Robinson BW, Schluter D (2000) Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. In: Mousseau TA, Sinervo B, Endler JA (eds) Adaptive genetic variation in the wild. Oxford University Press, Oxford, pp 65–94

Roulin A, Ducret B, Ravussin PA, Altwegg R (2003) Female colour polymorphism covaries with reproductive strategies in the tawny owl Strix aluco. J Avian Biol 34:393–401

Rowe L, Arnqvist G (2002) Sexually antagonistic coevolution in a mating system: combining experimental and comparative approaches to adress evolutionary processes. Evolution 56:754–767

Rowe L, Houle D (1996) The lek paradox and the capture of genetic variance by condition dependent traits. Proc R Soc Lond B 263:1415–1421

Rueffler C, Van Dooren TJM, Leimar O, Abrams PA (2006) Disruptive selection and then what? Trends Ecol Evol 21:238–245

Sanchez-Guillen RA, Van Gossum H, Rivera AC (2005) Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata:Coenagrionidae). Biol J Linn Soc 85:471–481

Schemske DW, Bierzychudek P (2001) Perspective: evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. Evolution 55:1269–1282

Schluter D (1998a) Ecological causes of speciation. In: Howard DJ, Berlocher SH (eds) Endless forms: species and speciation. Oxford University Press, Oxford, pp 114–129

Schluter D (1998b) Ecological speciation in postglacial fishes. In: Grant PR (ed) Evolution on islands. Oxford University Press, Oxford, pp 163–180

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Schluter D, Price T, Mooers AO, Ludwig D (1997) Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711

Shuster SM, Sassaman C (1997) Genetic interaction between male mating strategy and sex ratio in a marine isopod. Nature 388:373–377

Shuster SM, Wade MJ (2003) Mating systems and strategies. Princeton University Press, Princeton

Sinervo B, Lively CM (1996) The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380:240–243

Sinervo B, Svensson E, Comendant T (2000) Density cycles and an offspring quantity and quality game driven by natural selection. Nature 406:985–988

Smith TB (1993) Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*. Nature 363:618–6120

Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. Trends Ecol Evol 14:179–185

Svensson E, Sinervo B, Comendant T (2001) Density-dependent competition and selection on immune function in genetic lizard morphs. Proc Natl Acad Sci USA 98:12561–12565

Svensson EI, Abbott J (2005) Evolutionary dynamics and population biology of a polymorphic insect. J Evol Biol 18:1503–1514

Svensson EI, Abbott J, Hardling R (2005) Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. Am Nat 165:567–576

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man. Aldine, Chicago, pp 137–179

Turelli M, Barton NH, Coyne JA (2001) Theory and speciation. Trends Ecol Evol 16:330–343

Van Dooren TJM (1999) The evolutionary ecology of dominance-recessivity. J Theor Biol 198:519–532

Van Gossum H, Stoks R, De Bruyn L (2001) Reversible frequency-dependent switches in male mate choice. Proc R Soc Lond B 268:83–85

Vercken E, Massot M Sinervo B, Clobert J (2007) Colour variation and alternative reproductive strategies in females of the common lizard Lacerta vivipara. J Evol Biol 20:221–232

Waxman D, Gavrilets S (2005a) 20 questions on adaptive dynamics. J Evol Biol 18:1139–1154

Waxman D, Gavrilets S (2005b) Issues of terminology, gradient dynamics and the ease of sympatric speciation in Adaptive Dynamics. J Evol Biol 18:1214–1219

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford

Wright S (1969) Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago