Discrete genetic variation in mate choice and a condition-dependent preference function in the side-blotched lizard: implications for the formation and maintenance of coadapted gene complexes

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Variation in female preference functions, both genotypic and phenotypic, has been largely ignored in the literature, despite its implications to the maintenance of genetic variation in populations and the resolution of the “Lek paradox.” Polymorphic populations, such as in the side-blotched lizard, provide ideal study systems for its investigation, especially in the context of incipient processes of sympatric speciation. Females of the side blotch lizard exist in 2 genetically distinct morphs, yellow throated and orange throated, that experience disruptive selection for life history traits. Males express 3 throat color morphs, blue, orange, and yellow, that exhibit alternative strategies in intrasexual competition. We experimentally tested for female preference in triadic mate choice trials to identify the presence of discrete genetic and condition-dependent variation in female preference function. We found that females did in fact show genetic variation in preference for males but that females also operate a multicondition preference function dependent upon the genotype of the female and her state (number of clutches laid). Females exhibited positive assortative mating prior to the first clutch. However, prior to later clutches, orange females switched choice, preferring yellow males. These findings are discussed in relation to the maintenance of coadapted gene complexes within populations and the prevention of divergent directional selection (population bifurcation) by condition-dependent variation in mate choice. Key words: coadapted gene complex, condition dependent, disruptive selection, genetic variation, lek paradox, mate choice, polymorphism, side-blotched lizard, sympatric speciation. [Behav Ecol 18:304–310 (2007)]

Species with genetically distinct alternative strategies are useful model systems for investigating mechanisms for the formation and maintenance of coadapted gene complexes that serve as a requisite for processes of sympatric speciation (West-Eberhard 1979; Sinervo and Svensson 2002). To date, the majority of research on species that exhibit alternative strategies has focused on the role of intrasexual selection (Austad 1984; Gross 1996; Brockmann 2001), with few studies examining the impact of interfamilial selection (Fox et al. 2002; Morris et al. 2003). Male choice has far reaching implications for the maintenance of genetic polymorphisms, depending on the preference function employed by the choosing sex.

Male choice is expected to occur where the costs of choosiness are exceeded by the benefits accrued from exhibiting a preference. Two possible adaptive reasons exist for mate choice: 1) indirect benefits, the result of genetic benefits to offspring fitness. This can be further subdivided into a) the maintenance of genetic compatibility or the reduction of recombinational load that results from sexual reproduction (Tregenza and Wedell 2000), b) additive genetic benefits to offspring reproductive success (the “good genes” hypothesis; Williams 1966, p. 193), and c) selection for “arbitrary” male display traits, a nonadaptive sensory exploitation model (Fisher “runaway” hypothesis; Fisher 1930; Lande 1981; Kirkpatrick 1982); 2) direct benefits to the chooser, such as increased survivorship or fecundity (Reynolds and Cote 1995; Godin and Briggs 1996).

THE MAINTENANCE OF GENETIC COMPATIBILITY

When the viability of offspring is highly dependent upon genetic compatibilities within the genotype of the individual (Tregenza and Wedell 2000), coadapted gene complexes are built up under the action of correlational selection (Brodie 1992; Sinervo et al. 2001; Sinervo and Svensson 2002; Sinervo and Clobert 2003), and so recombination should not be a strong force in determining female preference functions. Alternatively, for unlinked traits, in linkage disequilibrium, any covariance will decay by 50% per generation (Pomiankowski and Sheridan 1994), leading to strong selection for assortative mating to maintain linkage disequilibrium. This axiom is central to theories of sympatric speciation by sexual selection (Dobzhansky 1940). Therefore, in populations that are inherently polymorphic and experience cyclical selection, we expect that females will exhibit positive assortative mating. Choice would be dependent upon not only male genotype but also on the genotype of the chooser.

ADDITIVE GENETIC BENEFITS TO OFFSPRING REPRODUCTIVE SUCCESS

If males exhibit genetic variation in alleles that contributes to offspring survivorship or reproductive success, indirect
selection will favor those females that adopt a preference function to sequester “good genes.” Selection for purely additive genetic fitness effects should be independent of the genetic background of the male unless differences in male display indicate differences in linked fitness loci (David et al. 2000). If preferences were independent of genotype, this would result in the break down of coadapted gene complexes built upon under the action of correlational selection. Directional selection for additive fitness loci will result in those loci reaching fixation. Direct selection would result in the loss of premating isolation and the break down of coadapted gene complexes.

DIRECT BENEFITS TO THE CHOOSER

Theoretical (Bulmer 1989; Kirkpatrick 1996) and empirical (Moller and Jennions 2001) evidence exists for direct selection on female preference as a stronger force than selection for indirect benefits. Although it has been suggested that any effect of a direct preference function acts independently of its genetic background (Servesto 2001), it is difficult to see how differentiation in female choice could evolve in sympathy under its control, unless an individual’s ability to absorb direct fitness costs has a heritable component. Differences in female “quality,” whether genetic or phenotypic, may lead to patterns of assortative mating, whereby selection favors females of lower quality mating with males that have a reduced impact on female fitness. Alternatively, condition-dependent female choice may be a possible mechanism to maintain genetic variation within a population. Therefore, it is possible that in polymorphic systems that experience strong indirect selection for assortative mating, variation in female abilities to weather direct fitness costs adds stochasticity to preference functions. Thus, maintaining genetic variation and the production of morph hybrid offspring.

In this study, we looked for the presence of positive assortative mating in light of the costs of recombinational load to offspring fitness. Throat color was used as a proxy for an individual’s genotype matings (e.g., orange or yellow). Throat color acts as an honest signal of relative male quality, being genetically hard wired and not condition dependent (Alonzo and Sinervo 2001). The most parsimonious explanation for the genetic architecture appears to be, a 3 allele, single locus model that simultaneously links male and female mating (Sinervo 2001). Color morphs in both sexes have an ovarian follicle size of 3–5 mm (Sinervo B, Comendant 2000). The action of positive assortative mating would also serve to maintain the linkage between what we refer to as strategic loci, which are any loci unlinked to the main morph determining locus, that serve to enhance the fitness of specific genotype combinations at the morph locus (Sinervo and Clobert 2003). Therefore, we predict that female phenotypes may exhibit variation in mate preference and can be used as a baseline to indicate the genetic control of female preference. The female has 2 basic mate choice strategies. The first is to maintain the integrity of the coadapted morph complex and participate in homologous genotype matings (e.g., orange × orange) (Sinervo 2001). The second is to adopt matings that enhance female lifetime reproductive success. Therefore, females may make context-dependent mate choice decisions.

THE STUDY SYSTEM

Inherent in populations of side-blotched lizards (Uta stansburiana) are 3 distinct throat color polymorphs (orange, blue, and yellow). Throat color acts as an honest signal of relative male quality, being genetically hard wired and not condition dependent (Alonzo and Sinervo 2001). The most parsimonious explanation for the genetic architecture appears to be, a 3 allele, single locus model that simultaneously links male and female mating (Sinervo 2001). Color morphs in both males and females are manifested as alternative reproductive strategies, which oscillate in frequency as a function of intrasexual competition in both sexes. Males cycle in a rock-paper-scissors game where orange usurpers invade blue male guarders, which are then invaded by a yellow sneaker strategy that is in turn invaded by the blue mate guarder strategy (Sinervo and Lively 1996; Zamudio and Sinervo 2000). This male cycle is intrinsically propagated (Bleay C, Sinervo B, in preparation) and entirely frequency dependent. Females are caught in a classic R-K game that is density dependent (Sinervo, Svensson, and Comendant 2000). Female reproductive allocation is genetically predetermined (Sinervo, Svensson, and Comendant 2000) in that orange females invest in quantity (larger clutches of smaller offspring) and yellow females invest in quality (smaller clutches of larger offspring). Female fitness or the survivorship of offspring is density dependent, where large hatchlings from yellow females gain an advantage in periods of high density (Sinervo, Svensson, and Comendant 2000). Conversely, orange females produce larger clutch sizes of smaller hatchlings that gain an advantage in periods of low density. In addition to the tight coupling of female reproductive strategies with throat color, an individual’s socially mediated stress response, humoral immunocompetence, and their ability to absorb the costs of reproduction (Svensson et al. 2001a, 2001b) are linked to female throat color. Yellow females showed an increased reduction in postlay mass as a function of clutch size compared with orange females (Svensson et al. 2001a). This suggests genetic differences between morphs in their ability to weather the impacts of reproductive allocation. These differences should manifest themselves in a female’s preference function, as direct fitness costs for a female’s progeny should have varying effects depending on the genotype of the female and the state of the population cycle (Alonzo and Sinervo 2001).

As a result of fluctuations in selection pressure, it is believed that a runaway process of correlational selection has resulted in the tight coupling of phenotypic traits to specific throat color alleles (Sinervo 2001; Sinervo et al. 2001). The action of positive assortative mating would also serve to maintain the linkage between what we refer to as strategic loci, which are any loci unlinked to the main morph determining locus, that serve to enhance the fitness of specific genotype combinations at the morph locus (Sinervo and Clobert 2003). Therefore, we predict that female phenotypes may exhibit variation in mate preference and can be used as a baseline to indicate the genetic control of female preference. The female has 2 basic mate choice strategies. The first is to maintain the integrity of the coadapted morph complex and participate in homologous genotype matings (e.g., orange × orange) (Sinervo 2001). The second is to adopt matings that enhance female lifetime reproductive success. Therefore, females may make context-dependent mate choice decisions.

METHODS

We designed an experiment to test for asymmetric patterns of intersexual selection in the side-blotched lizard. Genotype-specific maternal preferences were easy to examine because females of the side-blotched lizard exist in 2 morphs, orange throated and yellow throated. The presence of a single orange allele causes the female to adopt the r-strategy type. Therefore, genetic variation in mate preference was assigned on the basis of differences in female morph mate preference. The role of conditional effects was investigated by examining changes in mate preference prior to the first clutch and the second clutch. Females show a marked reduction in condition after the production of their first clutch of eggs (Svensson et al. 2001a).

In order to test for the presence of female mate preference, relative choice trials were carried out in a triadic choice chamber (Figure 1). During February, prior to the onset of the mating season, 47 female and 45 male lizards were removed from a population at Los Banos and brought into the lab. Females were housed in vivaria (approximately 20 l), given food ad libitum, with both light and heat on a 12-h cycle. They were then left for 5 days in order to acclimatize. When females reached a period of reproductive receptivity, determined by abdominal palpation (ovarian follicle size of 3–5 mm), they were presented with 3 males, one from each male morph, matched for size, in a relative preference trial. Extensive fieldwork over the course of 14 years indicates that courting females have an ovarian follicle size of 3–5 mm (Sinervo B, unpublished data).
RESULTS

Not all females exhibited acceptance displays (unambiguous choice, 24 trials) so discriminant function analysis using data from the unambiguous trials was employed to assign a choice to all other trials (ambiguous choice, 43 trials). Using discriminant function analysis is a more rigorous approach than simply using association time per se because, unlike the majority of studies of mate choice that use only one criterion (time), we have used independent indicators of choice (rejection and display) to determine the weight assigned to other behavioral indicators of choice. In the side-blotched lizard, we are fortunate enough that there are both a conspicuous rejection and acceptance display that are unambiguously indicative of choice, lack of these displays did not imply no choice. Discriminant function analysis works by determining the relationship between a dependent categorical variable and a number of continuous independent variables. This relationship is expressed in the form of a discriminant prediction equation that can then be used to assign individuals to a category based on their position in independent variable space. In total, 24 trials (13 first clutch, 11 second clutch) were unambiguous and so used in the discriminant function analysis. Independent variables were calculated as a relative value, proportionate to the total time of the trial or as proportionate incidence, and arcsine transformed. The time that a female spent in a particular male chamber, the residuals of female head bobs regressed on male head bobs and time, and the number of "tastes" were used as determinants for the model. Each variable was entered in a forward stepwise model until any subsequent contribution would be \( r < 0.01 \). Both time and residuals of female head bobs were retained in the model with a canonical correlation of 0.73 (Wilks' Lambda; \( F_{2,24} = 8.98, P < 0.005 \)). Two trials were misclassified; however, these were positives that were wrongly assigned, so any classification based on the discriminant function analysis was conservative. The forthcoming discriminant prediction equation was then used to assign a choice to females in the ambiguous trials so that the total sample size used in subsequent analyses was 45 females from the first clutch and 25 females from the second clutch.

There were a number of factors that may have contributed to observed patterns of female preference. Logistic regression of temperature (\( \chi^2 = 0.218 \), degrees of freedom [df] = 1, \( P > 0.05 \)), male mass (\( \chi^2 = 0.46, df = 1, P > 0.05 \)), male snout vent length (SVL) (\( \chi^2 = 1.79, df = 1, P > 0.05 \)), male body condition defined by the residuals of male mass on SVL (\( \chi^2 = 1.03, df = 1, P > 0.05 \)), and a chi-square of male chamber position were all found to have no significant effect on female preference. So as to remove any effect of pseudoreplication, both male and female IDs were entered into subsequent models as a random effect.

Nominal logistic regression was used to look for the effect of male phenotype and female state on the probability of acceptance by female morph. Both male morph and female state
had a significant effect on the pattern of choice for a particular female morph. In yellow-throated females (Figure 2A), there was a strong, significant, preference for yellow-throated males irrespective of female condition (Table 1). Orange females, on the other hand, exhibited a different pattern of choice (Figure 2B). There was a significant interaction between female state and preference for a particular male morph (Table 1). Prior to the first clutch, orange females showed a preference for orange-throated males, but this preference shifted toward yellow-throated males prior to the second clutch. A possible explanation for the shift in orange female preference as a function of female condition may be the reduced aggressiveness of yellow males (Figure 3). Reproduction has a dramatic effect on female condition; however, irrespective of the male, female side-blotched lizards exhibit genetic differences in the ability to weather the impact of reproduction.

Yellow females lost less weight (as a proportion of their pre–first clutch mass) as a result of laying their first clutch than did orange females (yellow mean = 0.27 ± 0.03, orange mean = 0.34 ± 0.01, t = 1.69, df = 65, P < 0.05).

That is to say, females may experience a direct fitness benefit by mating with less aggressive males for their second clutch when their body condition is greatly reduced. Yellow males did show significantly less activity (where activity is measured as the residuals of male head bobs from both proportionate time a female spends with a male and the proportion of head bobs a female displays toward a particular male) toward females than orange and blue males (F_{2,36} = 5.075, P < 0.05). Males that did not exhibit any head bobs during the trial were classified as null responses and removed from the analysis.

**DISCUSSION**

It was evident that female side-blotched lizards exhibit morph-specific variation in their preference function. Females mated assortatively, orange females exhibited a preference for orange males and yellow females preferred yellow males, prior to the first clutch. Furthermore, we also found evidence of a condition-dependent change in mate choice that was associated with the production of first and second clutches.

The difference in preference function, between female morphs, is inferred to be genetic. Although no measure of repeatability (Godin and Dugatkin 1995) or genetic correlations across generations (Houde 1992) were possible, given the highly heritable nature of throat color (Sinervo and Svensson 2002), female preference and throat color should be genetically linked. Evidence for the genetic control of mate preference in other species is strong (Houde 1992; Bakker

**Figure 2**

Incidence of female preference for male phenotypes. The solid line shows the expected frequency assuming random probability of preference at 0.33. (A) Yellow female preference and (B) Orange female preference.

**Figure 3**

The difference of yellow males from both orange and blue males. Where Activity is measured as the residuals of male head bobs from both proportionate time a female spends with a male and the proportion of head bobs a female displays toward a particular male.

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**Table 1**

The effects of male phenotype and female condition on the incidence of female preference by morph (yellow females and orange females)

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>df</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td><strong>Yellow females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full model</td>
<td>19.28</td>
<td>5</td>
<td>0.002**</td>
</tr>
<tr>
<td>Male phenotype</td>
<td>16.92</td>
<td>2</td>
<td>0.0002***</td>
</tr>
<tr>
<td>Clutch no.</td>
<td>0.007</td>
<td>1</td>
<td>0.933</td>
</tr>
<tr>
<td>Male phenotype x clutch no.</td>
<td>0.90</td>
<td>2</td>
<td>0.637</td>
</tr>
<tr>
<td><strong>Orange females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full model</td>
<td>14.82</td>
<td>5</td>
<td>0.011*</td>
</tr>
<tr>
<td>Male phenotype</td>
<td>0.86</td>
<td>2</td>
<td>0.651</td>
</tr>
<tr>
<td>Clutch no.</td>
<td>0.01</td>
<td>1</td>
<td>0.922</td>
</tr>
<tr>
<td>Male phenotype x clutch no.</td>
<td>8.97</td>
<td>2</td>
<td>0.011*</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.005, ***P < 0.0005.
with several examples of discrete variation in female preference function (Sappington and Taylor 1990; Fox et al. 2002; Morris et al. 2005). In systems that experience disruptive selection for fitness traits, such as female reproductive allocation in the side-blotched lizard (Sinervo, Svensson, and Comendant 2000; Svensson and Sinervo 2000), genetic variation is increased (Thoday 1972; Halliburton and Gall 1981; Sappington and Taylor 1990). Positive assortative mating, as exemplified by female preference functions in the side-blotched lizard, would act to reduce genetic diversity and so maintain the discrete nature of the genetic polymorphism. Assortative mating will reduce genetic variation in the broad sense by creating a number of discrete genetic combinations within the population.

Mate choice for assortative mating can be ascribed to indirect female fitness if offspring generated from between-morph matings lie at the antinode of a bimodal fitness distribution created by disruptive selection. Females may be choosing males on the basis of good genes (Williams 1966), yet this is dependent on the phenotype, and ultimately the background genetic architecture of the female. If females selected mates based purely on an additive good genes model, then female preference functions would be expected to resemble those of Alonzo and Sinervo (2001) where the optimal female preference switches between males of different morphs based on their relative fitness in the population, irrespective of female morph.

In our study, we also found that orange-throated females exhibit a state-dependent switch point in their preference function. Prior to the first clutch, orange females showed positive assortative mating, mating with orange males; however, prior to their second clutch, orange females switched, showing a preference for yellow males. The probability of switching mate preference was morph specific with yellow females showing no such adaptive behavior.

State-dependent variation in female choice strategies has received little attention in the literature. Theoreticians have understood the consequences of female state on reproductive decision making, but little empirical evidence exists in relation to mate choice decisions.

Age or timing of breeding in relation to the duration of the breeding season is known to alter female mate choice decisions (Backwell and Passmore 1996; Gray 1999; Moore PJ and Moore AJ 2001). Predictions are that females will become less choosy as their potential for future reproductive success diminishes (Gray 1999; Moore PJ and Moore AJ 2001), a random preference is expected. This was not found in the present study. Previous examples of state-dependent switches in female preference are mainly linked to changes in direct fitness costs (Koga et al. 1998; Luttbeg et al. 2001). Changes in predation pressure have resulted in female guppies changing their preference from brightly colored males to duller males in the presence of cichlid predators (Gong and Gibson 1996).

Females may exhibit differences in their abilities to weather direct fitness costs of reproduction that could have reciprocal effects on differences in preference function. Orange males were found to be more active, displaying more readily to females, and are known to have higher levels of testosterone making them more aggressive (Sinervo, Miles, et al. 2000). Females that mate with orange males may incur a higher direct cost to reproduction as a result of male harassment (Wilcox 1984; Rowe 1994; Muhlhauser and Blanckenhorn 2002). Classically, manifestations of variation in female choice in relation to male harassment are a function of female size, with smaller females unable to endure the costs of mating with larger males (Bourne 1993). Neck-biting behavior by males is a component of courtship and mating in the side-blotched lizard that has the potential to result in female injuries (Hiruki et al. 1993a, 1993b; Le Galliard et al. 2005). The probability of sustaining an injury of this type may therefore be increased with the aggressiveness of the male in combination with the reduced ability of the female to defend herself. In populations of the common lizard, Le Galliard et al. (2005) found that as the proportion of males in the population increased so did male-inflicted mating injuries along with reduced female survivorship and fecundity. Differences between females in respect to the impact of direct fitness costs may be a function of differences in condition generated by alternative reproductive allocation strategies. In the side-blotched lizard, the different male morphs may offer alternative fitness consequences such that a female can ameliorate any cost by picking and choosing her mate based on her condition.

Alternatively, it is possible that orange females switch choice of mate based on indirect fitness benefits (Lesna and Sabelis 1999; Alonzo and Sinervo 2001; van Gossum et al. 2001). Unpublished data (Sinervo B, personal communication) on male survivorship suggest that yellow males are recruited from the second clutch, with few surviving from the first clutch. Only orange and blue males from the first clutch survive into the next breeding season. Orange females may be sequestering yellow strategic loci for investment into males in the second clutch. If this is true, orange females should invest disproportionately in males on the second clutch.

CONCLUSIONS

Here it is impossible to distinguish between the alternative hypotheses for the proximate mechanisms of selection that promote mate choice. Whether it is good genes, a Fisherian runaway process, maintenance of genetic architecture, direct selection, or even sensory exploitation, the same patterns of covariance between preference trait and mate choice could be observed. What is shown is that there exists a great deal of variance in female choice within populations. Variation in female choice has the potential to maintain genetic variation in populations even in the presence of divergent directional selection. A standard population-wide optimum strategy does not exist. The optimum choice of mate is dependent upon the context, whether it is genotypic or phenotypic, in which a decision is made and can result in females switching preference to the extent that multiple optimal pairings within a population can exist. The number of optimum mates should increase exponentially as a function of genotype × genotype × environmental interactions to the extent that variation in both female mate choice and its reciprocal effects on male display trait could never be exhausted, even in the face of strong directional selection. Fitness traits are under continually fluctuating selective environments, with the effects of genes being context dependent, that context being the background genetic architecture with which it interacts, the background gene frequencies within the population, and environmental fluctuations in natural selection pressures. Stochastic, frequency dependent, density dependent, and epistatic interactions maintain genetic variation within populations. If females differ in the fitness consequences of sequestered "good genes," this will maintain genetic variation; as a result, epistatic interactions will be different between females causing females to select different males. The fact that female choice can be unrepeateable may indicate a large context dependent or conditional rule in female preference functions. In systems with alternative strategies, disruptive selection can result in concomitant selection for assortative mating; however, pleiotropic effects on condition-dependent preference functions can act to maintain genetic variation preventing reproductive isolation. Whether these preference functions manifest themselves in population mating patterns depends on the cost of choosiness (Slagsvold et al. 1988; Dawkins and...
REFERENCES


