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MOLECULAR EVIDENCE FOR SELECTION ON FEMALE COLOR POLYMORPHISM IN THE DAMSELFLY *ISCHNURA GRAELLSII*

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Abstract.—The significance of female color polymorphism in Odonata remains controversial despite many field studies. The importance of random factors (founder effects, genetic drift and migration) versus selective forces for the maintenance of this polymorphism is still discussed. In this study, we specifically test whether the female color polymorphism of *Ischnura graellsii* (Odonata, Coenagrionidae) is under selection in the wild. We compared the degree of genetic differentiation based on RAPD markers (assumed to be neutral) with the degree of differentiation based on color alleles. Weir and Cockerham's θ values showed a significant degree of population differentiation for both sets of loci (RAPD and color alleles) but the estimated degree of population differentiation (θ) was significantly greater for the set of RAPD loci. This result shows that some sort of selection contributes to the maintenance of similar color morph frequencies across the studied populations. Our results combined with those of previous field studies suggest that at least in some *I. graellsii* populations, density-dependent mechanisms might help to prevent the loss of this polymorphism but cannot explain the similarity in morph frequencies among populations.

Key words.—Damselflies, density-dependent selection, female color polymorphism, fertility, frequency-dependent selection, population structure.

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Several species of damselflies exhibit female-limited polychromatism, with two or more female morphs coexisting in natural populations. Typically, one of the morphs is brightly colored like the conspecific male (androchrome female), whereas the other phenotypes are more cryptic (gynochrome females; Cordero and Andrés 1996). This sex-restricted color polymorphism, in which one of the female morphs is male-colored is not restricted to Odonates; some butterfly and lizard species also have ''male-mimics'' and cryptic or Batesian mimic females (Cook et al. 1994; Forsman and Shiner 1995).

Ischnura graellsii is a small damselfly with three different female color morphs (one androchrome and two gynochromes: *infuscans* and *aurantiaca*), and several lines of indirect evidence suggest that female color polymorphism is under selection in this species. First, the frequencies of the morphs remain constant within populations and these frequencies are very similar across populations. Second, the existence of differences in the genetic basis of color morphs in different species suggests that this polymorphism could have evolved independently under similar selection pressures (Andrés and Cordero 1999), and third, within the genus *Ischnura* the existence of this polymorphism is related to the degree of polygamy (Robinson and Allgeyer 1996). Of the seventeen species reviewed in the latter study, all polymorphic species belong to polyandrous groups, although the females of monandrous species have only one color pattern.

Several adaptive hypotheses have been proposed to explain the maintenance of this color polymorphism in natural populations. Johnson (1975) proposed a postzygotic reproductive isolation mechanism as the main force maintaining variability in sympatric populations of closely related species with similar-looking gynochrome females. According to Johnson, gynochrome females are commonly involved in matings with males of other species (sterile matings), whereas androchrome females suffer a greater predation rate. However, this hypothesis cannot explain the persistence of this polymorphism in allopatric populations or in the absence of interspecific matings, which are extremely rare (Cordero and Andrés 1996).

As in butterflies and lizards, sexual conflict over mating has also been proposed as the main selective force maintaining female color polymorphism in damselflies. Robertson (1985) proposed a frequency-dependent selection mechanism for the maintenance of the different female color morphs in natural populations. By ''mimicking'' males, androchrome females avoid unnecessary (and costly) matings. This advantage would be counterbalanced by a frequency independent mechanism, such as more intense predation on androchrome females, due to their more conspicuous coloration.

Selective predation on common prey types could also maintain color polymorphism leading to a selective advantage of the rare morphs (Allen 1988), but this hypothesis can not explain why the color polymorphism is only restricted to one sex, unless there is a strong requirement for long-distance visual signaling amongst males, as occurs in some butterflies in which color polymorphism is also restricted to the females (Vane-Wright 1989). This long-distance signaling requirement could be important in territorial species (like some butterflies), but seems to be unnecessary for Ischnuran damselflies with ''scramble competition'' mating systems.

Fincke (1994) proposed a different frequency-dependent mechanism. If the most common female morph is the most

TABLE 1. Frequencies (%) of the female color morphs in the five Galician (northwest Spain) study populations; *aurantiaca* and *infuscans* are gynochrome morphs. Population density was measured as the number of males captured per minute.

Population	*N*	androchrome	*aurantiaca*	*infuscans*	Density
Castelo	42	11.90	11.90	76.19	1.04
Corrubedo	28	21.43	17.86	60.71	0.83
Campus	68	1.47	8.82	89.70	1.55
O Rosal	68	8.82	5.88	85.29	1.66
Lanzada	75	14.66	9.33	76.00	—

attractive to males, the polymorphism could be maintained by negative frequency-dependent selection. This hypothesis assumes that less frequent morphs enjoy a greater fitness by avoiding the costs of long copulations and/or male harassment as long as they remain relative rare.

Alternatively, Hinnekint (1987) proposed another explanation based on density-dependent selection mechanisms. At high densities (when the sex ratio is more biased towards males), the androchrome morphs might benefit by avoiding costly copulations, but at low densities some would not be able to mate. This situation would be the inverse for gynochrome females. The existence of temporal cyclic variation in population density would permit the different morphs to achieve an evolutionary equilibrium (Hinnekint and Dumont 1989; Cordero 1992).

Fincke (1994) concluded that neither the adaptiveness nor the mechanisms maintaining female polymorphism in natural populations of damselflies have been adequately demonstrated, and it is thus not possible to rule out random factors as the most important factors maintaining the color polymorphisms in damselflies.

When morph frequencies are relatively constant across populations (as is usually the case in *Ischnura graellsii,* see Table 1), it is difficult to differentiate between spatially uniform selection and high levels of migration between populations (Gillespie and Oxford 1998). An effective way to discriminate between these alternatives is to use the genetic structure between populations to generate a null model against which to test for selection (Cook 1992; Gillespie and Oxford 1998). This approach, for example, has recently been used to detect selection acting on color polymorphism in Hawaiian happy-face spiders (see Gillespie and Oxford 1998 and references therein).

Genetic differentiation between populations due to random factors (founder effects, genetic drift and migration) is reflected by the degree of population differentiation at the neutral set of loci. Thus, if the locus (or loci) in which we are interested is selectively neutral, the genetic differentiation at this locus should be similar to that obtained for the neutral markers. On the other hand, selection acting on a locus (loci) could either increase or decrease the degree of population differentiation relative to the neutral case (see Whitlock and McCauley 1999). Thus, a significant difference in the degree of differentiation between the ''target'' locus and the neutral markers would strongly suggest selection acting to maintain a different degree of population differentiation in the ''target'' locus. In this way, uniform selection acting over a wide area could decrease the expected value of population differentiation, whereas local divergent selection could increase it (Gillespie and Oxford 1998).

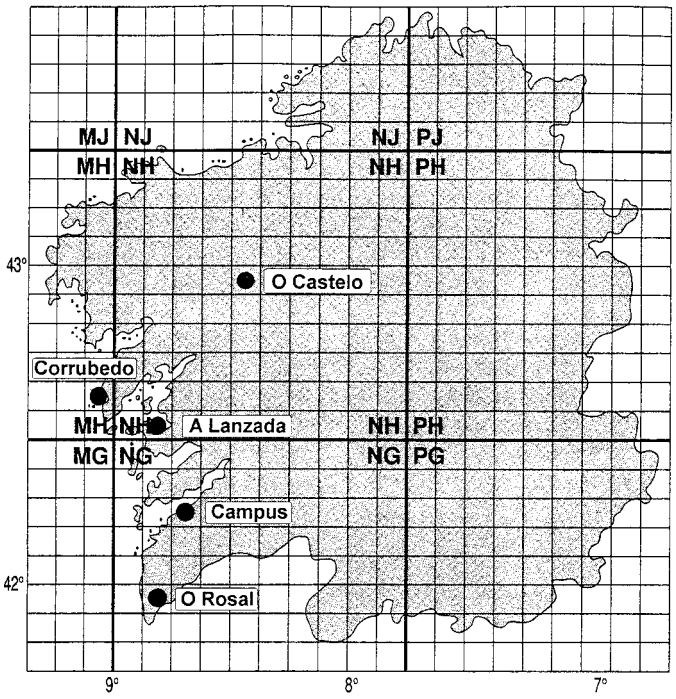

FIG. 1. Map of Galicia (northwest Spain) showing the location of each of the study populations. the squares are UTM coordinates of 10 × 10 km.

In this paper we use RAPD polymorphisms (assumed to be neutral) to assess the expected neutral degree of genetic differentiation between the studied populations and we compared it with the degree of population differentiation obtained for the female color polymorphism locus. We thus specifically test whether female color polymorphism in *Ischnura graellsii* is under selection in the wild. Our results are discussed in light of previous field studies.

METHODS

Studied Populations

In this paper we study five different *Ischnura graellsii* populations in Galicia (northwest Spain, see Fig. 1). These populations are separated from each other by large distances. All populations are large (more than one thousand individuals per generation) and inhabit permanent ponds with abundant vegetation (mainly *Potamogeton* sp.). Two of the study populations (A Lanzada and Corrubedo) are on the coast, less than 500 m from the shoreline. The most southern population, O Rosal, has an altitude of 50 m, while Castelo and Campus have altitudes of 250 m and 300 m, respectively. The population density was measured as the number of males captured per minute.

Color Morphs and Allele Frequencies

All populations were sampled between 6 and 17 July 1999, at the same time (13:00–18:00 h) and under the same weather conditions (sunny days and more than 25°C). In each pop-

ulation the captured animals were counted and released after marking them with a waterproof marker (Staedtler Pancolor) on a wing. A random subset of the captured males was kept for genetic analysis. Within each population, color morph frequencies (Table 1) were used to obtain estimates of allele frequencies for color morphs using knowledge about the genetic basis of female polychromatism. Color morphs are controlled by a single autosomal locus with three alleles with the expression restricted to the female. The three alleles are, p^a (androchrome), p^i (*infuscans*), and p^o (*aurantiaca*), with the dominance hierarchy $p^a > p^i > p^o$. Thus, the androchrome morph has three possible genotypes (p^ap^a, p^ap^i, p^ap^o), the morph *infuscans* two (p^ip^i, p^ip^o), whereas the *aurantiaca* morph has only one genotype (p^op^o; Cordero 1990).

Allelic frequencies for color morphs were calculated by maximum-likelihood estimates (see Hedrick 1985) on the assumption that the populations are in Hardy-Weinberg equilibrium.

Allele frequencies were used to calculate the genotype frequencies used as input to FSTAT 1.2 (Goudet 1995) for calculation of differentiation statistics.

Population Differentiation, Comparison of RAPDs, and Color Morph Alleles

We analysed RAPD polymorphisms to estimate allele frequency differentiation by making three assumptions: (1) RAPD products segregate in a dominant Mendelian fashion (Williams et al. 1990); (2) RAPD products are neutral (which allowed us to calculate expected heterozygosities), and (3) both—dominant and recessive alleles are assumed to be homologous in all individuals.

The genetic diversity was measured using Shannons diversity index, calculated as $H = -\Sigma p_i \log_2 p_i$, where p_i is the frequency of a given RAPD fragment. H was calculated for two different levels, the diversity within populations (H_S) and the total genetic diversity (H_T). Shannon's diversity index could be used to analyse the RAPD data because of its insensitively to the bias that could be introduced by the inability to detect heterozygous individuals (Dawson et al. 1995).

Estimates of the degree of genetic differentiation between populations (F_{ST}) were determined using Weir and Cockerham's (1984) θ, because this estimator corrects for unequal and small sample sizes. For the RAPD data set, calculations were made using the RAPDFST program of Black (1995). Means and 99.9% confident limits for θ were obtained by bootstrapping across loci. θ values for color alleles were obtained using FSTAT 1.2, as mentioned above.

To test if the obtained θ values were significantly greater than zero, we used a permutation analysis, which is the method that requires fewest assumptions (Giles and Goudet 1997). These analyses were made using FSTAT 1.2 by permutation of alleles among samples using 5000 random allele reallocations.

The test for significant differences between the two estimates of population subdivision was made by comparing the θ estimates for color alleles with the θ estimates and 99.9% confidence limits based on RAPD markers (see Gillespie and Oxford 1998).

RAPDs Analyses

Whole genomic DNA was extracted from adult thoracic muscles. DNA was purified by grinding tissue in liquid nitrogen, digesting with Tris HCL pH = 9.5 (10 mM), EDTA (10 mM), KCl (100 mM), sucrose (0.5M), mercapto-ethanol (0.1%), N-Laurosyl-sarcosine (2%), and extracted with phenol-chloroform 1:1 before ethanol precipitation. The concentration of the extracted DNA was estimated by comparing the band intensity of samples on 1% agarose gels to a known amount of DNA standard. DNA samples were stored in TE 1 × at a standardized concentration of 50 ng/μl.

Amplification was carried out in 25 μl reaction volume overlaid with mineral oil, using 10-mer oligonucleotide primers (Operon Technologies, Inc, Alameda, CA). The amplification conditions were optimized to allow amplification of the loci: 50 ng genomic DNA (1 μl), 1 unit (0.2 μl) of Taq DNA polymerase (Ecogen), 1 μl 10× reaction buffer (Ecogen, Barcelona, Spain), $MgCl_2$ (50 mM) 200(M each dCTP, dGTP, dTTP, dATP (Sigma dNTP mix), and 2 pmol of primer. PCR amplifications were done in an (Eppendorf (Hamburg, Germany)) Personal Mastercycler. The thermocycler was programmed for an initial denaturation step of 94°C for 5 min, followed by 45 cycles of 94°C for 1 min, 35°C for 1min, 72°C for 2min, and a final extension step of 72°C for 5 min. Reactions mixtures were separated on 1.5% agarose gels containing 0.75μl of ethidium bromide using 1× TAE running buffer.

Suitable primers were found by screening a total of 60 10-mer random primers (OPA01-OPA20, OPD01-OPD20, OPE01-OPE20, Operon Technologies, Inc.) on three representative individuals. We selected two of them (OPA-16, 5′-AGCCAGCGAA-3′ and OPA-17, 5'-GACCGCTTGT-3′) which amplified the highest number of polymorphic bands. This allowed us to reduce the total number of amplification assays required. These two primers produced a total of 38 reproducible polymorphic bands.

Collected males were used for RAPD analysis. Clear visible bands were scored manually for presence (1) or absence (0) from photographs of the gels. Differing band intensities were not taken into account to avoid errors introduced by competition among priming sites during the initial rounds of PCR (Bachmann 1997). Only bands reproducible in at least two independent amplification reactions were included in the data analyses.

RESULTS

The frequencies of female morphs showed a similar pattern among populations: *infuscans* females were always the most common morph, whereas the other gynochrome morph (*aurantiaca*) was the rarest, except for one of the populations (Campus). However, the frequencies of female morphs were significantly different among populations ($\chi^2 = 16.258$, df $= 8$, $p = 0.0388$, see Table 1).

The analysis of genetic variation based on RAPD markers revealed that the genetic diversity within populations ($H_S = 14.48$) accounts for most of the total genetic diversity ($H_T = 15.84$), while the genetic diversity among populations represented only a small proportion of the total diversity. The

TABLE 2. Genetic diversity (measured as Shannon's *H* values) based on 38 RAPD loci for the five *Ischnura graellsii* populations studied.

Population	Castelo	Corrubedo	Campus	O Rosal	Lanzada
H_S	14.54	14.94	14.83	13.53	14.47
mean H_S	14.46				
H_T	15.84				

extent of genetic diversity within each population was nearly identical (Table 2).

Frequencies of RAPD and color alleles are given in Table 3. These frequencies were used to estimate the degree of genetic differentiation among populations (Table 4). The degree of genetic differentiation among populations was significantly greater than zero for the RAPD data set ($P < 0.001$), and for the color locus ($P = 0.010$). However, the estimate of population differentiation averaged over all RAPD (neutral) markers was greater than the value based on female color morph alleles. This difference is statistically significant since the θ values based on color alleles do not overlap with the 99.9% confidence intervals for the RAPD θ value (Table 4).

DISCUSSION

Both color alleles and the set of RAPD alleles show significant genetic structure, but the frequencies for color alleles (and therefore the female morphs) were significantly more constant among populations than those for the RAPD set of loci. In other words, the degree of genetic differentiation between populations at the female color locus was significantly less than expected if color morph frequencies were only determined by random factors. This result could be explained if the polymorphism is maintained through female migration between populations or by some form of selection on the color locus to maintain similar morph frequencies over a wide area. The first explanation is ruled out by the fact that there was a high level of population differentiation at the RAPD data set, indicating that our populations are quite isolated and, therefore, there is a low migration rate between them. This conclusion is also supported by the long distance between the studied populations (see Fig. 1). The most plausible explanation for our results is that some sort of selection is acting to generate similar morph frequencies among populations (see Table 1), despite any environmental differences among them.

A possible weakness on the above results arises by the fact that we assumed that Hardy-Weinberg equilibrium exists in the studied populations. Nevertheless, at the color locus, this assumption is supported by two different lines of evidence. Firstly, within populations, females mated at random with respect to their coloration (Cordero 1992) and secondly, the estimate of population subdivision based on the frequency of female color morphs is similar to the estimate based on color alleles frequency (data not shown), suggesting that the deviation from Hardy-Weinberg (if any) is not important.

Different mechanisms of frequency- (see Robertson 1985; Fincke 1994) and density-dependent (Hinnekint 1987) selection have been proposed to explain the maintenance of female color polymorphism in Odonates.

TABLE 3. Allele frequencies of the two different sets of loci (color and RAPD loci) in the five *Ischnura graellsii* populations studied. For the set of RAPD loci ($n = 38$) we present the frequencies of the dominant allele. Each RAPD locus was named using the code of the primer used (A16 or A17) followed by a lower-case letter. The frequency of color alleles was calculated by MLE from the morph frequencies in the populations. All calculations assume that populations are at Hardy-Weinberg equilibrium.

Locus	Castelo	Corrubedo	Campus	O Rosal	Lanzada
RAPD	$n = 33$	$n = 33$	$n = 32$	$n = 35$	$n = 32$
A16a	0.000	0.051	0.016	0.000	0.016
A16b	0.000	0.000	0.048	0.000	0.016
A16c	0.018	0.106	0.048	0.000	0.048
A16d	0.134	0.317	0.229	0.000	0.190
A16e	0.094	0.106	0.081	0.122	0.190
A16f	0.433	0.317	0.363	0.414	0.441
A16g	0.074	0.394	0.339	0.172	0.152
A16h	0.293	0.184	0.171	0.000	0.081
A16i	0.319	0.204	0.271	0.172	0.048
A16j	0.537	0.342	0.171	0.345	0.363
A16k	0.373	0.317	0.567	0.263	0.532
A16l	0.134	0.423	0.271	0.493	0.414
A16m	0.094	0.163	0.065	0.090	0.099
A16n	0.000	0.069	0.048	0.074	0.099
A16o	0.500	0.087	0.171	0.324	0.099
A16p	0.402	0.817	0.567	0.244	0.694
A16q	0.155	0.144	0.209	0.189	0.152
A16r	0.114	0.069	0.134	0.090	0.065
A16s	0.402	0.204	0.229	0.493	0.363
A16t	0.622	0.423	0.646	0.283	0.694
A16u	0.000	0.017	0.032	0.207	0.016
A16v	0.094	0.124	0.048	0.189	0.152
A16w	0.094	0.247	0.134	0.244	0.229
A16x	1.000	0.124	0.363	0.831	0.250
A16y	0.221	0.204	0.209	0.106	0.152
A17a	0.155	0.034	0.032	0.029	0.032
A17b	0.319	0.293	0.250	0.553	0.250
A17c	0.345	0.144	0.099	0.172	0.171
A17d	0.345	0.204	0.081	0.244	0.048
A17e	0.221	0.517	0.532	1.000	0.315
A17f	0.134	0.087	0.190	0.59	0.229
A17g	0.198	0.204	0.209	0.189	0.209
A17h	0.577	0.817	0.750	1.000	0.605
A17i	0.345	0.144	0.152	0.090	0.229
A17j	0.433	0.204	0.065	0.225	0.081
A17k	0.433	0.317	0.500	0.391	0.363
A17l	0.319	0.592	0.414	0.283	0.293
A17m	0.134	0.106	0.363	0.368	0.081
Color	$n = 42$	$n = 28$	$n = 68$	$n = 68$	$n = 75$
p^a	0.061	0.113	0.073	0.045	0.076
p^i	0.593	0.464	0.695	0.712	0.618
p^0	0.345	0.423	0.297	0.242	0.305

TABLE 4. Comparison between RAPD and color allele based estimates of Weir and Cockerham's θ. For the RAPD dataset we present the arithmetic mean and 99.9% confidence intervals (in parentheses) after bootstrapping among loci. For the color locus we present the estimates for each allele as well as the arithmetic mean.

Dataset	θ
All RAPD loci	0.061 (0.060–0.063)
Color alleles:	
p^a	0.017
p^i	0.024
p^0	0.008
mean	0.016

Frequency-Dependent Selection

Frequency-dependent selection could be achieved by frequency-dependent mating success or predation rate. However, previous field studies have shown that although androchrome females are less attractive to males than gynochromes, they are found in copula as expected if the mating rate of females is independent of their coloration (Cordero 1992). Furthermore, the survivorship, measured as mean life span, of the different morphs was similar (Cordero 1992), suggesting that mortality factors are only weakly related to female coloration.

Color polymorphism could also be achieved by negative frequency-dependent selection if the less frequent morph enjoys a greater fitness by avoiding the costs of male harassment, as long as they remain relative rare (Fincke 1994). This hypothesis assumes that males are more attracted to the most common morph in the population (as seems to be the case in *I. elegans;* Van-Gossum et al. 1999, but see Cordero Rivera & Andrés 2000). However, in *I. graellsii, aurantiaca* females are always at low frequencies (see Table 1) and this morph appears to be as attractive to males as the most common morph, *infuscans* (see Cordero and Andrés, 1996).

On the other hand, under negative frequency-dependent selection with random mating and with fitness declining linearly with the frequency of each morph, all three morphs should have the same frequency at equilibrium (Bulmer 1994). However, Table 1 and previous fields studies showed (see Cordero and Egido 1998 as an example) that the frequencies of the morphs remain constant and far from the expected equilibrium frequencies under this kind of selection.

Density-Dependent Selection

Hinnekint (1987) proposed that female color polymorphism could be maintained by a density-dependent mechanism related to the cost of mating: at high densities androchrome females gain a selective advantage because males mistake them for other males, and thus they avoid superfluous matings, whereas at low densities there is the risk of no mating at all. This hypothesis assumes that androchrome females mate less often than gynochromes. However, field studies in *I. graellsii,* do not support this assumption. First, males that mate more than once do so randomly with respect to female coloration in both high and low density populations (Cordero 1992). Second, in a comparative study of the female mating frequency in four natural populations (two of them, O Rosal and Campus, also studied in this paper), Cordero and Egido (1998) found that androchromes did not mate less often than gynochromes in most populations. Third, while female mating probability increases with the density of males, this is true for all female morphs, and not only for androchromes as Hinnekint's hypothesis predicts (Cordero and Egido 1998).

Despite these results, Cordero and Egido (1998) also showed that the frequency of androchrome females increases with male density, suggesting the existence of other density-dependent costs and benefits for female morphs. Gynochrome females may suffer a decrease in fitness under high population densities as the result of male discrimination between morphs as potential mates, if greater male harassment towards them reduces their fitness by reducing their fecundity.

Density-dependent selection, however, could not explain why the frequencies of the morphs remained similar among populations unless densities (and their fluctuation patterns) were similar for all the studied populations, which seems highly improbable. Thus, although some kind of density-dependent selection might be involved in the maintenance of female color polymorphism in *I. graellsii,* some other selective mechanisms must also act to maintain similar morph frequencies over a wide area.

In conclusion, we provide evidence that some sort of selection mechanism is likely acting to maintain the female color polymorphism in *I. graellsii.* Our results combined with those of previous field studies suggest that, at least in some *I. graellsii* populations, density-dependent mechanisms, although not well established, might help to prevent the loss of this polymorphism but cannot explain the similarity in morph frequencies among populations. The interaction of density-dependent female fecundity and fertility, instead of density-dependent mating success (Hinnekint 1987) and negative frequency-dependent selection (the less frequent female morphs enjoying a greater fitness by avoiding the costs of male harassment; Fincke 1994) might be the clue to understanding this intriguing phenomenon.

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Literature Cited

Allen, J. A. 1988. Frequency dependent selection by predators. Phil. Trans. R. Soc. Lond. B 319:485–503.

Andrés, J. A., and A. Cordero. 1999. The inheritance of female color morphs in the damselfly *Ceriagrion tenellum* (Odonata, Coenagrionidae). Heredity 82:328–335.

Bachmann, K. 1997. DNA markers in plant biosystematic research. Opera Bot. 132:137–148.

Black IV, W. C. 1995. FORTRAN programs for the analysis of RAPD-PCR markers in populations. Colorado State University, Ft. Collins, CO.

Bulmer, M. 1994. Theoretical evolutionary ecology. Sinauer Associates Sunderlan, MA.

Cook, L. M. 1992. The neutral assumption and maintenance of color morph frequency in mangrove snails. Heredity 69:184–189.

Cook, S. E., J. G. Vernon, M. Bateson, and T. Guilford. 1994. Mate choice in the polymorphic African swallowtail butterfly, *Papilio dardanus*: male-like females may avoid sexual harassment. Anim. Behav. 47:389–397.

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

———. 1992. Density-dependent mating success and color polymorphism in the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). J. Anim. Ecol. 61:769–780.
Cordero, A., and J. A. Andrés. 1996. Color polymorphism in odonates: females that mimic males? J. Br. Dragonfly Soc. 12:50–60.
———. 2000. Estimating female morph frequencies and male mate preferences of polychromatic damselflies: a cautionary note. Anim. Behav. *In press.*
Cordero, A., and F. J. Egido. 1998. Mating frequency, population density and female polychromatism in the damselfly *Ischnura graellsii*: an analysis of four natural populations. Etología 6: 61–67.
Dawson, I. K., A. J. Simons, R. Waugh, and W. Powell. 1995. Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. Heredity 74: 10–18.
Fincke, O. 1994. Female color polymorphism in damselflies: Failure to reject reject the null hypothesis. Anim. Behav. 47:1249–1266.
Forsman, A., and R. Shiner. 1995. The adaptive significance of color pattern polymorphisms in the australian scincid lizard *Lampropholis-delicata.* Biol. J. Linn. Soc. 55:273–291.
Giles, B. E., and J. Goudet. 1997. Genetic differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. Am. Nat. 149:507–526.
Gillespie, R. M., and G. S. Oxford. 1998. Selection on the color polymorphism in Hawaiian happy-face spiders: evidence from genetic structure and temporal fluctuations. Evolution 52: 775–783.
Goudet, J. 1995. FSTAT vers. 1.2. A computer program to calculate *F*-statistics. J. Hered. 6:485–486.
Hedrick, P. W. 1985. Genetics of populations. Jones and Barlett Publishers, Boston, MA.
Hinnekint, B. O. N. 1987. Population dynamics of *Ischnura e. elegans* (Vander Linden) (Insecta: Odonata) with special reference to morphological color changes, female polymorphism, multiannual cycles and their influence on behaviour. Hydrobiologia 146:3–31.
Hinnekint, B. O. N., and H. J. Dumont. 1989. Multi-annual cycles in populations of *Ischnura e. elegans* induced by crowding and mediated by sexual aggression (Odonata: Coenagrionidae). Entomol. Gener. 14:161–166.
Johnson, C. 1975. Polymorphism and natural selection in Ischnuran damselflies. Evol. Theory 1:81–90.
Robertson, H. M. 1985. Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. Anim. Behav. 33:805–809.
Robinson, J. V., and R. Allgeyer. 1996. Covariation in life-history traits, demographics and behaviour in ischnuran damselflies: the evolution of monandry. Biol. J. Linn. Soc. 58:85–98.
Van Gossum, H. R. Stoks, E. Matthysen, F. Valck, and L. De Bruyn. 1999. Male choice for female color morphs in *Ischnura elegans* (Odonata, Coenagrionidae): testing the hypothesis. Anim. Behav. 57:1229–1232.
Vane-Wright, R. I. 1989. The role of pseudosexual selection in the evolution of butterflies color patterns Pp. 251–253 *in* I. R. Vane-Wright and P. R. Ackery, eds. The biology of butterflies. Princeton University Press, Princeton, NJ.
Weir, B. S., and Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Whitlock, M. C. and D. E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. Heredity 82: 117–125.
Williams, J. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbritary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531–6535.

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COPULATING WITH MULTIPLE MATES ENHANCES FEMALE FECUNDITY BUT NOT EGG-TO-ADULT SURVIVAL IN THE BRUCHID BEETLE *CALLOSOBRUCHUS MACULATUS*

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Abstract.—Postcopulatory sexual selection theory has come a long way since the evolutionary implications of sperm competition were first spelled out by Parker (1970). However, one of the most enduring questions remains: why do females copulate with multiple males? Here we show that females copulating with multiple males lay more eggs than those copulating repeatedly with the same male. We also show egg-to-adult survival to be more variable when females copulate multiply with different males and less variable when they copulate multiply with the same male. This supports the notion that egg-to-adult survival may depend on the genetic compatibility of males and females. However, pre-adult survival was highest when females copulated repeatedly with the same male rather than with different males. Thus, it would appear that polyandry in this species does not function to reduce the risk of embryo failure resulting from fertilization by genetically incompatible sperm.

Key words.—Callosobruchus maculatus, fecundity, genetic compatibility, polyandry.

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The recognition that copulation has costs (Keller and Reeve 1995) and that females frequently copulate with more than one male (Smith 1984; Birkhead and Møller 1998), requires that the function of polyandry be addressed. Whereas male reproductive success increases with each additional copulation (Bateman 1948), female reproductive benefits are much less obvious.

The possible benefits to females from copulating with multiple males fall into two broad categories: direct and indirect fitness benefits. Direct benefits include fertility insurance