ANIMAL BEHAVIOUR, 2002, **63**, 677–685
doi:10.1006/anbe.2001.1948, available online at http://www.idealibrary.com on IDEAL®

Evolution of female colour polymorphism in damselflies: testing the hypotheses

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(Received 23 June 2001; initial acceptance 2 August 2001; final acceptance 20 October 2001; MS. number: 6971)

The existence of several female colour morphs is a conspicuous characteristic of many damselflies that show one male-like (androchrome) and several nonmale-like (gynochrome) morphs. We tested several adaptive hypotheses and the null model for the maintenance of female polychromatism (one androchrome and two gynochromes) in the damselfly *Ceriagrion tenellum*. We tested the null model by comparing the degree of genetic differentiation between the colour locus and a set of 19 neutral RAPD loci in five populations. Our results indicate that selection is acting to maintain similar frequencies between populations at the colour locus. Using mark–recapture techniques we found that mating success is not dependent on female coloration. We tested the mimicry hypothesis by presenting live and dead models to males. Dead models were highly attractive irrespective of coloration. In contrast, with live models males could not distinguish between androchromes and other males, and were more attracted to gynochrome females. Despite this, within populations morph frequencies remained constant over time and mating was at random with respect to female coloration. However, there was a positive relationship between male density and androchrome frequency in a comparative study of eight populations. We discuss our results in the framework of sexual conflict theory and suggest that andro- and gynochrome females are using different strategies to control their number of matings. The different morphs might be maintained in a balanced polymorphism by a combination of density- and frequency-dependent mechanisms.

Intersexual conflicts arise when beneficial traits of one sex impose costs on the other (Parker 1979). In polyandrous insect species, male reproductive success (number of eggs fertilized) is an increasing function of male mating rate. However, females generally maximize their reproductive success (number of viable eggs produced) by mating only a few times (Arnqvist & Nilsson 2000). Thus, optimal mating rates differ between the sexes, giving rise to sexual conflict over the mating rate. This conflict is expected to be exacerbated in species where copulations and mate-guarding phases are long. In many odonates, long copulations as well as pre- and postmating guarding strategies have evolved as a consequence of intrasexual selection (Waage 1984; Cordero 1990; Sawada 1999; Andrés & Cordero Rivera 2000). These male adaptations to reduce sperm competition are potentially costly for females, and may thereby select for female traits that reduce these costs. For instance, females of many polyandrous odonates, whose mating systems are characterized by long copulation and guarding phases, occur as different morphs (Robinson & Allgeyer 1996). Typically, one of the coexisting morphs resembles a male to some extent (androchrome females), while the other morphs are usually cryptic (gynochrome females). Androchromes, by mimicking males, may avoid the fitness costs of male harassment and excessive mating rate (Robertson 1985; Hinnekint 1987; Cordero 1992).

If androchrome coloration increases female fitness, why do gynochrome females exist? It is unlikely that random factors (founder effects and migration) are responsible for the maintenance of female colour polymorphism in these species (Fincke 1994). In contrast, several lines of evidence (see Andrés et al. 2000) suggest that female coloration is an adaptive trait. One possibility is that the polymorphism is transient, with the androchrome morph having a selective advantage over the others. A second explanation is that it is a balanced polymorphism in which the different morphs are at a stable equilibrium. Several hypotheses have been proposed to explain how the equilibrium in female colour

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0003–3472/02/$35.00/0

Table 1. Frequencies (%) of the different female morphs in the five populations included in the molecular analyses

Population, UTM coordinate	*N*	Androchrome	Gynochromes	
			typica	*melanogastrum*
A Castiñeira, 29TNH4362	67	10.45	68.66	20.90
As Graveiras, 29TNH4059	53	7.55	58.49	33.96
Pozo de Lago, 29TNG7995	58	1.72	65.52	32.76
Maus de Salas, 29TNG8842	80	28.75	62.50	8.75
O Castelo, 29TNH2663	49	10.20	53.06	36.73

polymorphism is maintained and all assume that sexual conflict over the mating rate is the main force behind each potential proximate mechanism.

First, the density-dependent hypothesis (Hinnekint 1987) suggests that sexual conflict over the mating rate and the existence of temporal variation in population density are the most important forces allowing the coexistence of different female morphs (Hinnekint & Dumont 1989; Cordero 1992). This may arise because, at low population densities, gynochrome females have a greater reproductive success, since some androchrome females would not achieve any matings. Conversely, at high densities, gynochrome females have a lower reproductive success as a result of higher fitness costs associated with male harassment and long copulations (Hinnekint 1987). The existence of temporal cyclic variation in population density would permit the different morphs to achieve an evolutionary equilibrium (Hinnekint & Dumont 1989).

A second set of hypotheses suggests that one of a variety of different frequency-dependent mechanisms is involved in the maintenance of this polymorphism. First, the advantage of androchrome females (i.e. reduced male harassment and avoidance of superfluous copulations) might be counteracted by more intense predation on this morph, owing to their conspicuous coloration, but only if they remain the rarest morph (Robertson 1985). Second, different morphs may be maintained solely by sexual conflict (Andrés & Cordero Rivera 2001; Sherratt 2001; Sirot & Brockmann 2001). If the most common morph is most attractive to males, the polymorphism could be maintained by negative frequency-dependent selection (learned mate recognition hypothesis, Fincke 1994; Miller & Fincke 1999). Under this hypothesis there is no need for any morph to resemble the conspecific male. However, in a formal model (signal detection model), Sherratt (2001) has shown that the different morphs could be at equilibrium if androchrome females are more conspicuous to males, and that males will learn to mate with androchromes as the frequency of androchromes rises.

We assessed the role of selection in the maintenance of the female morphs in the small red damselfly, *Ceriagrion tenellum*, a model species which shows the typical reproductive behaviour of nonterritorial, polyandrous damselflies. We did this by using the genetic structure between populations at a neutral set of loci to generate a null model against which to test for selection. This method has been applied effectively to detect selection acting on colour polymorphisms in several organisms (mangrove snails, *Littoraria pallescens*: Cook 1992; happy-face spiders, *Theridion grallator*: Gillespie & Oxford 1998; damselflies: Andrés et al. 2000).

We also performed a series of field experiments that helped us to recognize what kind of selection pressures allow the coexistence of more than one female morph. Since different mechanisms for the maintenance of this polymorphism predict different male mating preferences, we first analysed these preferences. If androchrome females are indeed male mimics (Robertson 1985; Hinnekint 1987; Cordero 1992), mating attempts should be more frequent with gynochrome than androchrome females. In contrast, the number of mating attempts among gynochrome morphs should be about the same. If female attractiveness is frequency dependent (Fincke 1994; Miller & Fincke 1999), then the number of mating attempts with any given morph should be (positively) related to its frequency in the population. In addition, we obtained estimates of the lifetime mating success of the different morphs. If one morph has a selective advantage over the others, populations are not in equilibrium, and we therefore predicted differential lifetime mating success among female morphs. Alternatively, if populations are in equilibrium (i.e. if there is a balanced polymorphism) female lifetime mating success is predicted to be independent of their coloration. Finally, in a comparative study across populations, we assessed the possible relationship between population density, operational sex ratio and morph frequencies predicted by some of the hypotheses (Hinnekint 1987; Sherratt 2001).

METHODS

RAPDs versus Colour Alleles: Testing the Null Hypothesis

We studied five *C. tenellum* populations from Galicia, northwest Spain (Table 1). All populations were sampled between 9 and 24 July 1999, at the same time (1200–1500 hours) and under the same weather conditions (sunny days and above 25°C).

An effective way to assess the role of selection in the maintenance of colour polymorphisms is to compare the genetic differentiation between populations at the colour locus with the degree of differentiation at a neutral set of loci (Cook 1992; Oxford & Gillespie 1998; Andrés et al. 2000). We used the method described by Andrés et al.

(2000), in which the degree of genetic differentiation at the colour locus is compared with a set of RAPD loci that are used to assess the degree of neutral genetic differentiation between populations. This test is based on the idea that selection acting on a locus could either increase or decrease the degree of population differentiation relative to the neutral case (Whitlock & McCauley 1999). Thus, any significant deviation in the degree of differentiation between the neutral markers and the colour locus would strongly suggest that selection is acting on the latter.

The extraction, quantification and amplification of the genomic DNA for the RAPD analyses was done following the protocol previously described for the damselfly *Ischnura graellsii* (Andrés et al. 2000). Suitable primers were found by screening a total of 60 10-mer random primers (Operon Technologies, Inc.) on three individuals of each of the populations studied. We selected one of the primers (OPA-17), which amplified a total of 19 polymorphic loci. Clear visible bands were scored for presence or absence from photographs of the gels. Differing band intensities were not taken into account (Bachmann 1997) and only bands reproducible in at least two independent amplifications were included in the analyses. We assumed that RAPD products segregate in a dominant Mendelian fashion (Williams et al. 1990) and that they are neutral with respect to population structure, that is, they are at Hardy–Weinberg equilibrium within populations (for details see Andrés et al. 2000).

Within each population we used colour morph frequencies to obtain estimates of allele frequencies using our knowledge of the genetic basis of the colour polymorphism in this species. In *C. tenellum*, female colour morphs are controlled by a single autosomal locus with three alleles (p^a, androchrome; p^t, *typica*; p^m, *melanogastrum*) with the dominance hierarchy $p^t > p^m > p^a$ (Andrés & Cordero 1999). Therefore, *typica* females have three possible genotypes (p^tp^t, p^tp^m, p^tp^a), *melanogastrum* females two (p^mp^m, p^mp^a), and androchrome females only one (p^ap^a). Allele frequencies were estimated by maximum likelihood methods (Hedrick 1985). Genotype frequencies were calculated from these estimates and used as input to FSTAT 1.2 (Goudet 1995) for calculation of differentiation statistics. For both data sets, the colour locus and the 19 neutral loci, we used Weir & Cockerham's (1984) θ to estimate the genetic differentiation between populations. The 99.9% confidence limits for θ at the RAPD set of loci were obtained by bootstrapping among loci.

Are Androchromes Male Mimics?

We tested the assumption that males are unable to distinguish between androchrome females and other males with a successive (single-trial) presentation experiment, that is, we presented only one morph at a time to the males. The single presentation was preferred over simultaneous presentation because it corresponds more closely to encounter patterns in nature (Cordero Rivera & Andrés 2001). Since model behaviour may be as important as coloration in male mate preferences (Cordero et al. 1998), we did two presentation experiments. First, pinned models were presented to 120 males. Second, live tethered specimens were presented to a further 219 males (see Cordero et al. 1998 for details of methodology). We carried out both experiments in the A Castiñeira population, during July 1995 and July–August 1997. The males used in this experiment were all sexually mature. At the end of each presentation trial, we marked individual males with a black spot on one of their wings to ensure we used each individual only once. The experiment was always conducted under sunny conditions. We classified male behaviour in three categories: an approach was defined as a male coming within 5 cm of a model, a contact as the male making any nonsexual physical contact with the model, and a copulation attempt as a male attempting to initiate a tandem with the female by curling his abdomen and trying to grasp her prothorax (see Cordero et al. 1998)

Lifetime Mating Success and Body Size

We estimated lifetime mating success from a mark–recapture experiment carried out during August–September 1995. In total, we marked 657 sexually mature females by writing a number on one of their wings with an indelible pen. Marked individuals were tracked daily, for an average of 5.5 h, by one or two observers and their activity (mating, ovipositing, perching, etc.) was noted at each sighting (for a full description of the methods see Andrés & Cordero 1998 and Andrés & Cordero Rivera 2001).

We tested differences in the lifetime mating success of female morphs in two ways. First, using Kruskal–Wallis tests followed by sequential Bonferroni corrections we analysed whether the mean interval between matings or the mean number of matings per female (standardized by either number of days alive or number of visits to the mating area) differed between morphs. Second, we divided lifetime mating success into three multiplicative components: life span, lifetime number of visits to the pond (where copulations take place) and number of matings per visit. The relative importance for lifetime mating success of each of the components was calculated with the method described by Brown (1988). This method does not take into account the variance caused by unmated individuals. However, in a separate analysis, with the same data set, we found that for all female morphs, 88% of the total variance in mating success was due to mated females and there was no significant difference between morphs in the proportion of females that did not mate (androchromes: 1/31; *melanogastrum*: 0/56; *typica*: 3/149; $\chi^2_2=1.452$, $P=0.484$).

We measured body size of the females from head to tip of the abdomen to the nearest 0.1 mm using a digital calliper.

Male Density and Androchrome Frequency

Hinnekint's (1987) density-dependent hypothesis predicts that the frequency of androchrome females should be positively correlated with population density.

We tested this prediction in eight populations in northwest Spain (Alligal de Candamil, Barra, A Castiñeira, As Graveiras, O Castelo, Goián, Maus de Salas and Pozo de Lago). In each population, we estimated male density as the number of males captured per min. Both morph frequencies and male density were estimated at the same time (1300–1700 hours) under sunny weather conditions. The relationship between these two variables was studied with isotonic regression, a method that allows a relaxation of the assumptions of linear regression and to test simply for the presence of a consistent increase (or decrease) in the dependent variable as a function of the rank order of the values of an independent variable (Sokal & Rohlf 1995).

Ethical Note

Ceriagrion tenellum is a very common species in Galicia. In this region, there are many populations of this small damselfly, the majority of which have several thousand individuals during the flying season. All experiments and captures were done with permission of the regional government (Xunta de Galicia).

For the DNA analyses, a sample of 50–55 individuals (all males) was taken at each of the studied populations. All individuals were killed rapidly by freezing (− 20°C). Our estimates of population size suggest that in each of these populations there were at least 4000 individuals during the flying season. So the sizes of these populations were not affected by our experiment.

For the presentation experiments, we used 42 models (30 live tethered and 12 pinned). Live models were presented to six or seven focal males over a period of 10–15 min. After the presentations, all the individuals were released. Live models were fastened with a nylon filament around the thorax. We killed dead models by pressing the thorax for 5–6 s. The total number of individuals used as models is an insignificant percentage of the population (see above).

For our mark–recapture experiment, individuals were captured with an insect net and marked by writing a number with an indelible pen on one of their wings. This technique has been applied in several long-term studies in damselflies and as far as we know has no adverse effects (which is a critical assumption of the mark–recapture methodology).

Statistical tests are two tailed unless stated otherwise.

RESULTS

Is Female Coloration a Neutral Trait?

As in other damselflies, despite the ecological differences between populations, the frequencies of female morphs showed a broadly similar pattern across populations (Table 1). In the populations studied *typica* females were the commonest, while androchrome females were the less frequent morph, excepting one population where *melanogastrum* females were rarer than androchromes. Although morph frequencies differed significantly between populations (χ^2_8=38.893, P<0.001), by removing the population of Maus de Salas this difference is no longer significant (χ^2_7=8.13, P=0.23).

We detected a significant genetic structure for the colour locus as well as for the set of neutral loci (permutation tests: P<0.01). The degree of genetic differentiation at the colour locus (θ) was significantly less than that expected by the neutral (RAPD) markers, since the estimate for the colour locus does not overlap with the 99.9% confidence interval of the θ value calculated after bootstrapping among the 19 neutral loci (Table 2).

Table 2. Comparison between RAPD and colour allele-based estimates of Weir & Cockerham's (1984) θ

Data set	θ
All RAPD loci	0.142 (0.130–0.154)
Colour alleles:	0.073
p^t	0.115
p^m	0.102
p^a	0.001

For the RAPD data set we present the arithmetic mean and 99.9% confidence intervals (in parentheses) after bootstrapping among loci. For the colour locus we present the estimates for each allele and the arithmetic mean.

Are Androchromes Male Mimics?

Table 3 shows the results of the single-trial experiment. For the statistical analyses, categories were pooled as follows: sexual (i.e. copulation attempts) and nonsexual responses (approached and contact categories of Table 3). Those males that did not respond to the models were excluded from the analyses. The results of the single-trial experiments with live models indicate that males preferred gynochrome (*melanogastrum* and *typica*) females over androchrome females, (χ^2_1=9.358, P<0.001). However, males responded in the same way to the two gynochrome morphs (χ^2_1=1.221, P=0.269). Similarly, males were not more attracted to androchrome females than to other males (χ^2_1=1.480, P=0.224). When pinned models were used (Table 3), the results were completely different: all models were highly attractive and males did not show any kind of preference (gynochrome versus androchrome females: χ^2_1=0.767, P=0.381; *typica* versus *melanogastrum*: χ^2_1=0.003, P=0.957; males versus androchrome females: χ^2_1=0.563, P=0.453).

Lifetime Mating Success and Body Size

In several species of damselflies, regardless of male mating preferences, mating occurs at random with respect to female phenotype (coloration, Andrés & Cordero Rivera 2001). To test this idea, we performed two different analyses. First, we compared the observed frequencies of the different female morphs seen in copula (82 androchromes:168 *melanogastrum*:407 *typica*) with

Table 3. Response of sexually mature *Ceriagrion tenellum* males to different live and pinned models

Model	*N*	Male response (%)			
		Approached	Contact	Copulation attempt	No response
Alive					
Male	30	40.0	20.0	13.3	26.7
Androchrome	59	18.6	15.2	16.9	49.1
melanogastrum	68	10.3	13.2	35.3	41.2
typica	62	11.3	6.4	45.2	37.1
Pinned					
Male	30	0.0	0.0	76.6	23.4
Androchrome	30	3.3	3.3	63.3	30.1
melanogastrum	30	0.0	0.0	73.3	26.7
typica	30	0.0	0.0	80.0	20.0

the expected frequencies obtained from the relative proportions of each morph in the population (75:155:369). This analysis revealed that males mated at random with respect to female coloration (χ^2_2=0.034, P=0.983). This is not surprising since both samples are almost identical (most of the females were marked in copula). To avoid this problem, we performed a second analysis in which we used a sample of 77 marked males that obtained two copulations. In this analysis (Table 4) the observed frequencies of the different double matings were compared with the expected frequencies if males mated at random with respect to female coloration. Again, there were no significant differences between the observed and the expected frequencies (χ^2_5=5.06, P=0.409). This analysis has a power of 0.80 to detect a size effect (w) of 0.41 with alpha 0.05.

Table 5 shows various estimates of mating success by female morphs. Including all marked females in the analyses, there were no differences between morphs in the number of matings. This is still true if the number of matings is standardized by the observed life span or by the number of female visits to the reproductive area. In this analysis nonresighted females were assigned a life span of 1 and mating success 0 or 1, depending on whether they were mating on the day they were marked. Taking into account only resighted females, our results suggest that androchrome females mated less often than gynochrome females (post hoc planned comparisons test: P<0.01), while there were no differences between the two gynochrome phenotypes (post hoc planned comparisons test: P=0.124). However, this difference disappears when the number of matings is standardized by female life span or number of visits to the reproductive area. Since the majority of the females included in the above analyses mated at least once (i.e. were marked in copula), it could be argued that these estimates of lifetime mating success are potentially biased. To avoid this problem, in 1996 we marked newly emerged females. Again, our results showed that lifetime mating success was not related to female coloration (Kruskal–Wallis test: χ^2_2=0.36, P=0.835).

The analysis of the contribution of different components of variance in female mating success (Tables 6 and 7) reveals that for all female morphs the variances in life span and the number of visits to the reproductive area were greater than the variance in female mating success. Consequently, these two single components and their product were the most important sources of variation in female mating success.

When the date of marking was controlled for, there were no significant differences in body length between female morphs during 2 consecutive years (ANCOVA: 1995: androchromes: $\bar{X} \pm$ SE=33.67 ± 0.13 mm; *typica*: 33.77 ± 0.06; *melanogastrum*: 33.56 ± 0.09; $F_{2,656}$=0.364, P=0.695; 1996: androchromes: 33.91 ± 0.34; *typica*: 34.43 ± 0.06; *melanogastrum*: 34.32 ± 0.04; $F_{2,816}$=1.973, P=0.139).

Table 4. Comparison between the observed and expected frequencies of double matings if males mate at random with respect to female coloration

	Observed frequency	Expected frequency
Androchrome–Androchrome	0	1.11
Androchrome–*melanogastrum*	3	4.80
Androchrome–*typica*	13	11.46
Melanogastrum–melanogastrum	4	5.20
Melanogastrum–typica	32	24.82
typica–typica	25	29.59

Data are from a mark–recapture experiment carried out on the A Castiñeira population during August–September 1995.

Male Density and Androchrome Frequency

The androchrome frequency in eight populations increased with male density (estimated as the number of males captured per min (Fig. 1).

DISCUSSION

We found evidence that the female morphs are not neutral to selection. Androchrome females indeed mimicked males and the attractiveness of a given

Table 5. Different estimates ($\bar{X}$±SE) of female mating success

Estimate	Androchrome	*melanogastrum*	*typica*	*P*
All females				
Matings/female	1.29±0.03 (82)	1.34±0.02(168)	1.32±0.01 (407)	0.454
Matings/day	0.70±0.04 (82)	0.72±0.03(168)	0.69±0.02 (407)	0.715
Matings/visit	0.83±0.03 (82)	0.89±0.02(168)	0.87±0.01 (407)	0.864
Resighted females				
Matings/female	1.87±0.19 (31)	2.46±0.13 (56)	2.20±0.07 (149)	0.007
Matings/day	0.41±0.05 (31)	0.38±0.08 (56)	0.39±0.02 (149)	0.148
Matings/visit	0.76±0.05 (31)	0.89±0.02 (56)	0.89±0.02 (149)	0.110
Copulation interval*	2.64±0.06 (31)	3.60±0.44 (82)	3.60±0.26 (182)	0.367
Copulation interval†	2.90±0.67 (22)	3.75±0.45 (49)	3.75±0.28 (125)	0.385

The *P* values are those of Kruskal–Wallis tests before the sequential Bonferroni correction was applied. Sample sizes are given in parentheses.
*The estimate of the interval between copulations, in days, calculated considering each interval as an independent event.
†The estimate of the interval between copulations, in days, calculated by averaging the intervals for each female.

gynochrome morph seemed to be independent of its frequency. However, mating was at random with respect to female coloration. That is, androchrome females mated as frequently as gynochrome morphs. As a result, the lifetime mating success of the females was only weakly related to female coloration. Among populations, high densities were correlated with a male-biased operational sex ratio (unpublished data) and, the higher the density, the higher the frequency of androchrome females in the population.

Table 6. Means and variances of the individual components of female mating success and their products

Component	Mean	Variance	G	G′
Androchrome				
L	6.966	24.447	0.503	170.91
V	0.536	0.110	0.382	129.88
C	0.738	0.067	0.123	41.91
LV	2.460	0.809	0.133	45.35
LC	5.237	17.522	0.617	209.47
VC	0.424	0.093	0.519	176.24
LVC	1.930	1.098	0.294	100
melanogastrum				
L	9.035	33.340	0.408	244.08
V	0.433	0.072	0.388	232.02
C	0.892	0.033	0.414	24.78
LV	2.767	1.017	0.132	79.37
LC	8.120	30.825	0.467	279.36
VC	0.378	0.057	0.404	241.73
LVC	2.464	1.016	0.167	100
typica				
L	8.212	28.085	0.416	296.07
V	0.434	0.072	0.382	271.88
C	0.910	0.042	0.051	36.44
LV	2.506	0.748	0.119	84.69
LC	7.312	23.187	0.433	308.31
VC	0.398	0.073	0.461	328.07
LVC	2.253	0.714	0.140	100

L=life span; V=number of visits to the reproductive area per day alive; C=number of matings per visit to the reproductive area. G is the variance of individual variables and their products (LV, LC and VC) after the individual variables have been divided by their means. G′ gives the values of the percentage of variance in mating success (LVC) accounted for by the individual variables or their products.

The degree of genetic differentiation was lower at the colour locus than at the RAPD loci. This result indicates that the frequencies of the colour alleles (and therefore morph frequencies) are more similar among populations than expected by the neutral model. That is, the genetic differentiation between populations at the colour locus was less than that expected if the colour morph frequencies were maintained only by founder effects, genetic drift and migration. The most plausible explanation for this lack of matching in the degree of genetic differentiation between the colour locus and the neutral markers is that some sort of selection is acting at the former locus (Andrés et al. 2000). Because different selection pressures could either increase or decrease the degree of genetic differentiation in relation to the neutral model (Charlesworth et al. 1997; Whitlock & McCauley 1999), the pattern of this difference is also very informative (Gillespie & Oxford 1998; Andrés et al. 2000). Our result strongly suggests that selection is acting over a wide area to maintain similar morph frequencies among the populations studied. A possible weakness in the interpretation of this result arises from the contrasting degree of genetic differentiation between the colour locus and so the neutral markers may be the effect of different mutation rates. However, this result seems to be robust since it is in agreement with previous findings in *Ischnura graellsii*, a small damselfly of the same family in which females are also polymorphic (Andrés et al. 2000).

Hence, if female morphs are not neutral to selection, what kind of selective forces are acting to maintain this polymorphism? One possible explanation is that it is a transient polymorphism in which the androchrome morph has a selective advantage over the others. However, at least three lines of evidence suggest that female morphs are in a balanced polymorphism. First, the frequencies of the morphs remain constant over time (Andrés & Cordero 1999). Second, as we discussed above, the observed pattern of genetic differentiation at the colour locus suggests that some sort of selection is

Table 7. Downward partition (Brown 1988): percentage contribution of the components of lifetime reproductive success to variation in mating success (LCV) in the different female morphs

	Androchrome			*melanogastrum*			*typica*		
	L	V	C	L	V	C	L	V	C
L	170.91			244.08			296.07		
V	−255.44	129.88		−396.74	232.03		−483.25	271.88	
C	−3.35	4.44	41.91	10.49	−15.08	24.78	−24.20	19.75	36.44
LVC	11.64			0.426			−16.69		

See Table 6 for definitions of L, V, C.

responsible for the coexistence of the different morphs. Third, female lifetime reproductive success seems to be independent of their coloration. For all the morphs, life span and the number of visits to the reproductive area were the most important components of lifetime mating success. The number of visits to the pond and the mating frequencies were similar for all female morphs and, as we have previously shown, there are no significant differences between morphs in survivorship or recapture rates during the reproductive period (Andrés & Cordero Rivera 2001). Consequently, we did not find any difference in mating success between morphs. Body size, a strong correlate of fecundity in odonates (Banks & Thompson 1987; Cordero 1991), did not differ between morphs in the field, suggesting that reproductive success (number of eggs laid) is likely to be similar among morphs. These results are therefore in disagreement with the transient polymorphism hypothesis.

So what are the mechanisms that maintain this balanced polymorphism? Sexual conflict over the mating rate has been proposed as one of the main selective forces

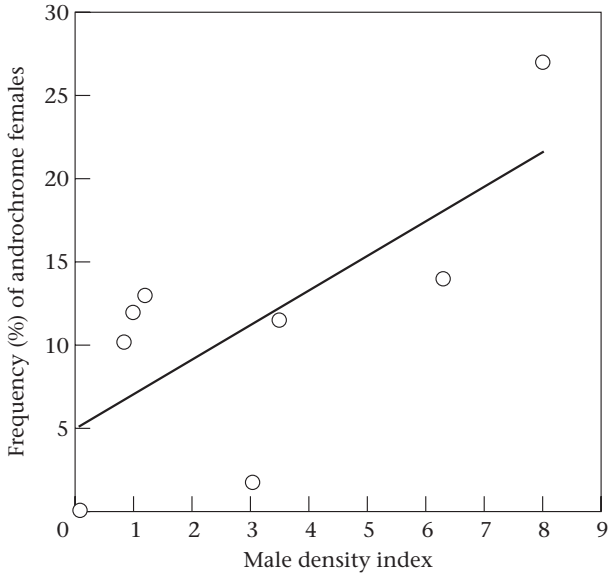

Figure 1. The relationship between male density (estimated as the number of males captured per min) and androchrome frequency in eight natural populations (χ^2_1=0.855, P=0.018, one tailed).

maintaining female colour polymorphism in several organisms (Hinnekint 1987; Fincke 1994; Andrés & Cordero Rivera 2001; Sherratt 2001; Sirot & Brockmann 2001). Different mechanisms have been proposed (see Introduction) to explain the coexistence of the different female colorations. According to some authors, conflict over the remating rate acts together with differential predation pressures to maintain the polymorphism (Robertson 1985; Hinnekint & Dumont 1989; Grether & Grey 1996). However, as we have shown, neither the mating frequency nor the survival probability differs between female morphs.

Other authors suggest that males are more attracted to the commonest female morph in the population (Miller & Fincke 1999; Van Gossum et al. 1999, 2001; but see Cordero Rivera & Andrés 2001). This hypothesis proposes that females suffer a cost from excessive matings and that this cost is frequency dependent. One of the key predictions of this hypothesis is that morph attractiveness increases as a function of its frequency. Our results do not support this prediction. First, within populations the morph frequencies at equilibrium deviated strongly from equality. Equality is the only stable equilibrium if there is negative frequency-dependent selection and fitness diminishes linearly with morph frequency (see also Andrés et al. 2000). Second, regardless of the low frequency of the *melanogastrum* females, this morph was as attractive as the most common morph (*typica*).

We have found that androchromes are a good imitation of males, and that some unidentified behavioural cues are affecting male recognition of female morphs, because dead lures are highly attractive irrespective of coloration. The same result has been found previously in a related species (Cordero et al. 1998; Cordero Rivera & Andrés 2001). This is important because it shows that males are using behaviour rather than colour (or a combination of both) to decide if a mating should be attempted with a given individual.

One clear result that emerges from this study and a review of previous work (Cordero Rivera & Andrés 2001) is that males show a preference for gynochromes when tested with single or paired presentations, but nevertheless mate at random with regard to female morph. The mimicry and the learned mate recognition hypotheses are based on differences of coloration between females, but it is clear that female behaviour is the main factor. Males

are probably detecting subtle behavioural differences between females, which suggests females can control their mating rate by behavioural means, even if males show clear preferences (Sirot & Brockmann 2001; H. Van Gossum, personal communication).

Male adaptations to reduce sperm competition (i.e. long copulations and guarding phases) as well as male harassment are likely to be costly for females. These, coupled with variations in population densities, might explain the existence of this polymorphism. Among populations, the frequency of androchrome females was correlated with male density. This result strongly suggests that, within populations, density is one of the mechanisms controlling the frequency of the female morphs. This 'density effect' has been shown in several polymorphic damselflies (Forbes et al. 1995; Cordero Rivera & Egido Pérez 1998), and therefore seems a robust finding. However, because high densities were associated with a sex ratio biased towards males (personal observation), it is possible to argue that the sex ratio of the population, rather than the density, is the variable responsible for the increase in the frequency of the androchrome females. A recent model suggests that, independently of population density, the equilibrium frequency for androchrome females increases when the operational sex ratio is more biased towards males (Sherratt 2001). Unfortunately, with the experimental approach used here it is impossible to disentangle the independent effects of a biased sex ratio or of population density in the equilibrium frequency of androchrome females.

In conclusion, our data support the idea that female morphs in *C. tenellum* are not selectively neutral. Higher densities are correlated with higher androchrome frequencies, which suggests that density is a key factor. Nevertheless, our results indicate that the maintenance of this polymorphism is not achieved through differential female mating frequency, as previous density-dependent models suggest (Hinnekint 1987). A frequency-dependent mechanism (i.e. learned mate recognition hypothesis, Miller & Fincke 1999) is also at variance with some of our results. A signal detection model (Sherratt 2001) predicts that this polymorphism is maintained by male mimicry coupled with a higher encounter rate of androchromes with males. Our results neither support nor reject this possible mechanism. Future work should measure male–female encounter rates, and test for male response at contrasting androchrome frequencies and population densities.

Acknowledgments

The molecular part of this paper was funded by a research project from the Spanish Ministry of Science and Culture to AC (PB97-0379). We thank T. Morrow, F. Johansson and L. Rowe for their helpful comments and criticisms on the manuscript.

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