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SHORT COMMUNICATIONS

A COMPARISON OF STRUCTURAL SIZE AND CONDITION IN TWO FEMALE MORPHS OF THE DAMSELFLY *NEHALENNIA IRENE* (HAGEN) (ZYGOPTERA: COENAGRIONIDAE)

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Female colour polymorphism in coenagrionid damselflies is genetically determined for the 4 spp. so far studied. No size differences between ♀ morphs have been reported. In another sp., size differences between morphs do exist but the genetic basis of the polymorphism has not been explored. In *N. irene*, 2 ♀ morphs exist: one is similar to the ♂ in both colour and pattern (the androchrome), whereas the other differs from the ♂ (the more common gynochrome). No differences are shown in lengths of wing, femur or tarsus between androchromes and gynochromes, nor any differences in multivariate estimates of size or in wet mass corrected for these size estimates were found. The study controls for time of season, which is known to influence the size of emerging temperate damselflies. The results concur with results from other spp. where the genetic basis of colour polymorphism is known.

INTRODUCTION

In many odonates, particularly coenagrionid damselflies, two or more female types exist in single populations (CORBET, 1999). One female morph is coloured and, depending on the species, patterned like the male, whereas the other(s) is different from the male in colour and pattern. The male-like female has been variously termed andromorph, homeochrome, or androchrome: the latter term being more accurate (CORDERO, 1990a; cf. HILTON, 1987). The other female(s) is usually the typical phenotype encountered in the population (but see FORBES, 1994) and has been termed the gynomorph, heterochrome, or gynochrome. These different female types are not due to age-related colour differences, which are also common among odonates (CORBET, 1999).

In the four species of damselflies where inheritance of colour polymorphism has been studied, morph type appears determined by simple Mendelian genetics. For instance, both *Ischnura demorsa* (Hag.) and *I. damula* Calv., androchromes (cf. andromorphs; JOHNSON, 1975) are homozygous recessive, with a single autosomal locus showing sex-linked expression. Whereas gynochromes are heterozygous or homozygous dominant, and males occur as all three genotypes, but are monomorphic (JOHNSON, 1975). In *I. graellsii* (Ramb.) there are three female coloration patterns that appear determined by three alleles at one autosomal locus. Androchromes are heterozygous or homozygous dominant (three possible genotypes), whereas one gynochromic female is homozygous recessive while the other is either homozygous recessive or heterozygous dominant if the other gynochromic allele is present (two possible genotypes). In *Ceriagrion tenellum* (de Vill.), the genetic basis for polychromatism is a composite of the two mechanisms thus far described. There are three female colour types and three alleles at a single autosomal locus with sex-linked expression, but the androchromic allele is recessive (ANDRÉS & CORDERO, 1999).

Female-limited polychromatism in damselflies so far described represents a genetic polymorphism, which may be under selection (ANDRÉS & CORDERO RIVERA, 2000; ANDRÉS et al., 2000), perhaps through social interactions in a frequency or density-dependent manner (CORDERO, 1989; FORBES et al., 1995; SHERRATT, 2001; but see FINCKE, 1994a, 1994b). Many studies have described behavioural differences between morphs, such as differential attention/harassment from mate-searching conspecific or heterospecific males (JOHNSON, 1975; ROBERTSON, 1985; CORDERO, 1990b; DE MARCHI, 1990; FORBES, 1991; FINCKE, 1994a; FORBES et al., 1997). Other researchers have reported different mating or mate rejection behaviours (ROBERTSON, 1985; FORBES et al., 1997; CORDERO RIVERA & Pérez, 1998; CORDERO et al., 1998; SIROT & BROCKMANN, 2001). Also, authors have described differential representation of one morph at sites with lower versus high population density (CORDERO, 1989; FORBES et al., 1995). But whether such differences actually translate into differences in average fitness of morphs at sites remains largely debated, making female-limited polychromatism in damselflies an ideal subject area for testing the alternatives of neutrality (FINCKE, 1994a) versus evolutionary stable states (GROSS, 1996). SHERRATT & FORBES (2001) argued that selection for aposematism of males may explain divergence in coloration of the sexes of damselflies and this same selection may also account for female-limited polymorphism as a secondary phenomenon. It is clear, though, that the social interactions mediated by males, thought to account for differential selection on morphs, are themselves flexible (MILLER & FINCKE, 1999; VAN GOSSUM et al., 2001).

To obtain a wider appreciation of selection that might be acting on the morphs, one must compare morphs against other attributes such as body size or condition. There are at least three reasons why such comparisons are important. First, many hypotheses posit that androchromes are actually male mimics (JOHNSON, 1975; ROBERTSON, 1985) or are at least not recognized as easily as females if they are the minority morph

(FORBES, 1994; FINCKE, 1994a). Evidence of convergence on male size or shape, in addition to similar coloration, pattern and behaviour (cf. FORBES et al., 1997) would suggest an even greater degree of apparent mimicry, if not functional mimicry. Second, differences in size could be indicate a condition-dependent strategy for a given species, rather than genetic polymorphism for morph type per se. Condition-dependent strategies are widespread in nature (GROSS, 1996), and could occur in some damselflies, even though evidence to date suggests genetic polymorphism in the norm (references cited above). Third, differences in condition could be a consequence of different selection on female morphs at particular sites, suggesting that further research at those sites should examine whether evolutionary stable states have been disrupted and are returning to equilibrium via fitness differences between morphs.

Here, we test whether two female morphs of *Nehalennia irene* (Hag.) differ in structural size or mass correcting for structural size. We adopt the terminology of androchromic and gynochromic females for our study species. In *N. irene*, only two female morphs have been reported. CALVERT (1913) observed a male-like female in this species and, since then, coloration and pattern of both morphs have been detailed (FORBES et al., 1995). The androchromic female not only differs in pattern and coloration from the gynochromic female, which makes up = 70% of the females at any particular study site (FORBES et al., 1995), but these morphs also differ in behaviour (FORBES et al., 1997). The androchromic females show certain male behaviours as well as greater expression of apparent mate rejection behaviours, although receptivity differences between colour morphs were not controlled (FORBES et al., 1997; cf. ROBERTSON, 1985). As in other studies (summarised by FINCKE, 1994b), males tend to direct more attention toward the gynochromic female, although recent studies have shown that experience with the less abundant morph may alter male mate-searching behaviours (VAN GOSSUM et al., 2001). Finally, although genetic markers in *N. irene* have been studied (WONG et al., 2001), markers relating to morph type have not been found (A. Wong, unpubl. data), and no study has yet tested the hypothesis that male-like females converge on male morphology in particular traits or in multivariate combinations of traits.

MATERIAL AND METHODS

Nehalennia irene is a small damselfly (body length 25-28 mm; CANNINGS & STUART, 1977) which is ubiquitous in most of Canada (WALKER, 1953). It begins emergence in the first week of June at our study location, but the reproductive season can last till mid-August. This species generally has a very short teneral period (< 24 hours) (WALKER, 1953), but even teneral females can be distinguished to chromatype based on pattern.

Our working hypothesis is that male-like females may differ from typical females and demonstrate more male-like morphology in multivariate space. To this end, we measured wing lengths of similar numbers of males, male-like females and typical females from one study location over three days. We did this because size declines with season in many temperate damselflies (FORBES & BAKER, 1991; CORBET, 1999; JOHANSSON & ROWE, 1999) and we wanted to control for any such variation. Additionally, we measured femur and tarsal lengths and recorded wet mass, as detailed below. Finally, we also examined wing cell asymmetry for each wing pair. Fluctuating asymmetry, including wing cell asymmetry, has been associated with various metrics of fitness and stressful conditions in other damselflies (e.g. HARVEY & WALSH, 1993; CÓRDOBA-AGUILAR, 1995; BONN et al., 1996; HARDERSON & WRATTEN, 1998; HARDERSON, 2000; but see LEUNG & FORBES, 1997).

N. irene were collected from June 26 - June 28, 2001 at Indian Lake Bight located 1 km from the Queen's University Biological Station near Chaffey's Lock, Ontario, Canada (44°34' N, 79°15' W). Females were identified as either androchrome (with distinctive male-like triangular patch of pale blue on abdominal segment VIII and a pair of dark spots on segment IX) or gynochrome (without distinctive male-like characteristics on segments VIII and IX) (following FORBES et al. 1995). On each trip, individuals were collected using sweep nets and put in transport cages (30 × 30 × 40 cm). In the laboratory, damselflies were measured for wet mass (to the nearest 0.0001 g using Mettler AE100 Digital Scale). Wing length from the nodus to the pterostigma on the right forewing was also measured (to the nearest 0.01 mm; Mitutoyo digital calipers), as was cell asymmetry of all wings (based on wing-cell counts between the nodus and pterostigmata on the left and right forewings and hindwings). Finally, the right metathoracic leg of each damselfly was mounted and preserved in Glycerol on a microscope slide. The last tarsal segment and the femur were measured using a micrometer (to the nearest 0.0001 mm; Graticules LTD, Tonbridge, Kent England) on a Leitz Laborlux K microscope (at 25 × and 10 ×, respectively). In some instances, the last tarsal segment could not be measured, as tarsal morphology was abnormal (see FORBES & BAKER, 1989).

RESULTS AND DISCUSSION

We collected 56 males, 54 male-like females and 47 typical females over three days. Due to partial damage of some individuals during processing, not all metrics could be obtained for each individual, accounting for slight differences in sample sizes using univariate tests. We first compared males and the two female morphs, hereafter termed sex/morph for differences in univariate measures of morphology. Using a series of one way ANOVAs, we found that sex/morph accounted for significant variation in wing lengths ($F_{2,153} = 28.7, P < 0.0001$). This result was due to the fact that males had smaller wings on average (10.1 ± 0.075 mm, mean ± SD) than either androchromic (10.8 ± 0.076 mm) or gynochromic females (10.8 ± 0.081 mm); which were statistically indistinguishable with respect to wing length ($F_{1,99} = 0.19, P = 0.66$). With respect to wet mass, sex/morph again accounted for significant variation ($F_{2,154} = 59.3, P < 0.0001$) because males were significantly lighter on average (8.7 ± 0.3 mg) than either androchromic (12.7 ± 0.3 mg) or gynochromic females (12.7 ± 0.3 mg). Again, the two female morphs did not differ with respect to wet mass ($F_{1,99} = 0.005, P = 0.94$). For femur lengths, a similar pattern was observed, as males had smaller femora (2.66 ± 0.017 mm) compared to either androchromic (2.71 ± 0.017 mm) or gynochromic females (2.74 ± 0.018 mm) ($F_{2,153} = 5.22, P < 0.01$). Finally, sex/morph did not account for significant variation in tarsi, as male tarsi (averaging 0.73 ± 0.006 mm) were comparable to tarsal length of both female colour morphs (both averaging 0.75; $F_{1,144} = 2.43, P = 0.09$). Taken together, males have shorter wings, are lighter, and have shorter femora than females, but do not differ from females in tarsal lengths. Additionally, female morphs were similar in all metrics, as reported above for wing lengths and wet mass and as seen for femur ($F_{1,99} = 1.52, P = 0.21$) and tarsal lengths ($F_{1,99} = 0.0047, P = 0.94$).

However, females could differ in relations between characters, or relations between mass and overall structural size. To test this, we first did a Principal Components Analysis on the three commensurable characters (i.e. excluding wet mass). Wing, femur, and tarsus lengths all loaded positively and strongly on the first axis (loadings > 0.45, representing structural size), which accounted for 61% of the overall variation in the data. As expected, sex/morph accounted for significant variation in the derived estimate of structural size or PC1 scores ($F_{2,144} = 11.6$, $P < 0.0001$), but here again this was due to males averaging lower scores (-0.70 ± 0.18) than either androchromic (0.29 ± 0.17) or gynochromic females (0.43 ± 0.19), which were themselves statistically indistinguishable. Using an ANCOVA, we found that androchromes and gynochromic females showed the same positive linear relation between wet mass and our estimate of structural size. In fact, only PC1 was a significant predictor of wet mass ($F_{1,99} = 22.7$, $P < 0.001$), whereas colour morph was not ($F_{1,96} = 0.28$, $P = 0.77$).

Thus, it appears that female morphs are similar with respect to particular characters, multivariate estimates of structural size, or wet mass correcting for such estimates of size. In other words, androchromes and gynochromes appear the same size and in the same condition, while still differing from males in three of four metrics.

As mentioned, some researchers have shown that fluctuating asymmetry measures relate to damselfly fitness or metrics of stress (see references above). Wing cell asymmetry has been used in this context, and can act as measure of developmental instability and perhaps condition before actual morph expression during larval-teneral development. We found that absolute forewing asymmetry ranged from 0 to 3 for females, as did absolute hindwing asymmetry. Forty-nine percent of 54 androchromic females had symmetrical forewings as compared to 48% of 47 gynochromic females ($\chi^2 = 0.006$, d.f. = 1, $P = 0.94$, in comparing whether symmetry versus some degree of asymmetry was contingent on female morph). Fifty seven percent of androchromic females had symmetrical hindwings as compared to ca. 62% of gynochromic females ($\chi^2 = 0.19$, d.f. = 1, $P = 0.66$). Clearly, female morphs did not differ in wing cell asymmetry measures.

At present, we have no evidence that androchromic females differ from gynochromic females in any measures of size or condition. This lack of difference in structural size is similar to that found in other zygopteran female morphs; such as in *Enallagma boreale* Sel. (wing length and head width; FORBES, 1994), *Ischnura graellsii* (body length; CORDERO, 1992), or *Coenagrion puella* L. (wing length and mass; THOMPSON, 1989). Therefore, there is limited evidence for condition-dependent polychromatism in *N. irene*. If it is a genetic polymorphism, then there are no pleiotropic effects on body size, nor are the alleles that control polychromatism linked to alleles that affect body size. Little convergence on male morphology by androchromes suggests that apparent or functional mimicry is limited to coloration and/or pattern in this species. In comparison with our findings, CORDERO et al. (1998) found that *I. elegans* (Vander L.) androchromes were larger on average than either gynochrome. This is perplexing since it could not be explained by differences in emergence dates, which as mentioned before, are correlated with size. However, in that species the genetic basis of the polymorphism is still unknown, and that androchromes are larger again suggests little convergence on male morphology, since males are still smaller than females. Future work on *N. irene* should disrupt densities or morph ratios at particular sites, and observe whether changes in morph condition or metrics of fitness appear. Such studies are currently being planned.

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