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Morph-Specific Fecundity and Egg Size in the Female-Dimorphic Damselfly *Ischnura senegalensis*

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Females of coenagrionid damselflies exhibit color dimorphism, consisting of an andromorph and a gynomorph. This study compared reproductive traits between the female morphs in both field-captured and laboratory-reared females of the female-dimorphic damselfly *Ischnura senegalensis*. No difference was found in the onset of egg development between the morphs. The andromorphs developed significantly smaller mature eggs and had significantly more immature eggs than the gynomorphs. These results suggest that the andromorphs are r-strategists (high fecundity with small eggs), whereas the gynomorphs are K-strategists (low fecundity with large eggs). Fecundity and egg size might determine the quantity and quality of the offspring, respectively, indicating that morph-specific reproductive traits would contribute to the overall fitness of each female morph, and consequently be key factors affecting morph frequency in a population.

**Key words:** damselfly, egg size, fecundity, female polymorphism, K-strategy, reproductive strategy, r-strategy, egg production

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**INTRODUCTION**

Female polymorphism within a population is an outcome of sexually antagonistic coevolution (Hayashi et al., 2007). In damselflies, one morph is characterized by a male-like color (andromorph), while the other morph exhibits cryptic coloration (gynomorph) (Robertson, 1985). Theoretical models explaining the maintenance of multiple female morphs in animals have focused on the negative frequency-dependent selection derived from intersexual interactions (Van Gossum et al., 2008), assuming that female body color might be subject to selection. In the damselfly species *Ischnura elegans*, female color polymorphism is maintained under balancing selection derived from selective male mating harassment (e.g., Svensson and Abbott, 2005). Because males prefer to mate with the common morph rather than the rare morph (Van Gossum et al., 1999, Takahashi and Watanabe, 2009), the intensity of male harassment to each female morph is correlated with the morph frequency (Van Gossum et al., 2005), leading to negative frequency-dependent selection for female morphs.

Differences in morph frequency among local populations have been reported in several damselfly species, including *Nehalennia irene* (Van Gossum et al., 2007), *I. elegans* (Sánchez-Guillén et al., 2005; Hammers and Van Gossum, 2008), and *I. senegalensis* (Takahashi and Watanabe, 2009). Phenotypic differences between the morphs have been detected in behavioral responses to mating attempts by males (Robertson, 1985; Sirot and Brockmann, 2001), microhabitat selection by adults (Van Gossum et al., 2001), the dispersal ability of adults (Abbott and Svensson, 2005), and the body shape of adults (Abbott and Svensson, 2008; Abbott and Gosden, 2009). Although differences in reproductive strategy between the morphs are key factors affecting morph frequencies in a population in female polymorphic species, because reproductive strategy potentially contributes to the fitness of the females (Joop et al., 2007; Sinervo et al., 2001), little information regarding the reproductive traits of each morph in damselflies has been reported. In the present study, we compared reproductive strategies between the morphs in the female-dimorphic damselfly *I. senegalensis* from the perspectives of fecundity, egg production, and egg size.

**MATERIAL AND METHODS**

**Reproductive traits of wild females**

Both female morphs were sampled by netting on 25 May 2009 from a population in the city of Tsuchiura, Ibaraki Prefecture (36°2’29.22”N, 140°8’50.55”E), in the warm-temperate zone of Japan. In all, 26 andromorphs and 34 gynomorphs were captured from 1000 to 1100. All were put into a cool box immediately after capture to inhibit their activity and were taken to a laboratory, where the age and the lengths of the abdomen and hind wing were recorded. The damselflies were classified into two age classes (sexually immature and mature) by the color of the thorax and abdomen. All females were dissected under a stereomicroscope, and the total number of ovarioles in the right and left ovaries was counted. For sexually mature females, the length and width of three eggs randomly sampled from each female were measured with a micrometer. We classified eggs in ovaries into three categories (mature, sub-mature, and immature), in accordance with Watanabe and Matsu’ura (2006). Mature eggs were yellow with a crystalline eggshell and seemed to be large enough for oviposition. Immature eggs were transparent and tiny. Sub-mature eggs were white and intermediate in size between mature and immature eggs. For sexually immature females, the number of immature eggs in each of three randomly sampled ovarioles was counted.
Reproductive traits of laboratory-reared females

To obtain test imagos, each female morph was allowed to artificially oviposit in the laboratory. Sexually mature females were captured from the same population at Tsuchiura as mentioned above and also from a population at Tsukuba, Ibaraki Prefecture (36°28.19’N, 140°34.79’E) from May to June, 2006–2008. The females were placed individually in plastic cups (120 mm in diameter, 60 mm high) for several days, with wet filter paper as an oviposition substrate. Eggs laid on the filter paper were kept in a petri dish filled with water at room temperature. Hatched larvae were maintained individually in plastic bottles (3.5 cm in diameter, 5.8 cm high) in the laboratory and fed Artemia salina or Tubifex spp. according to the larval body size. A twig approximately 10 cm in length was provided in each bottle as a support for the emergence of the final instar larva. To reduce any physical damage to the wings, newly emerged individuals were not handled until they started to show flight activity. An individual identification code was marked on the right hind-wing with a fine felt-tipped pen. The lengths of the abdomen and hind wings were measured with electronic calipers (accuracy, 0.1 mm). Sexes were separated in respective flying cages (40 cm × 40 cm × 50 cm) consisting of wooden frames covered by polypropylene mesh (mesh size 1 mm). Grass blades were put in a conical flask and placed in each cage to provide perch sites. The cages were placed by the window in the laboratory for exposure to direct sunlight. Adults fed freely on cultured fruit flies. To increase the humidity as well as to supply water, we misted the inside of each cage 2 or 3 times a day.

To compare fecundity between the morphs, young virgin females (0–3 days old) were decapitated and dissected under a stereomicroscope. After the ovary was gently detached from the abdomen, the ovarioles in the right and left ovaries were counted. The number of immature eggs in five randomly sampled ovarioles was counted for each female, and the total number of immature eggs was then calculated.

Virgin females 0–15 days old were decapitated and dissected under a stereomicroscope, and the mature eggs they contained were counted. The lengths and widths of three mature eggs randomly sampled from each female 5–9 days old were measured with a micrometer.

Statistical analysis

Statistical analyses were performed using R version 2.9.0 (R Development Core Team, 2009). All values are presented as means ± standard error. Differences between the morphs for each trait were analyzed with a generalized linear model (GLM) assuming a Gaussian or Poisson error distribution.

RESULTS

Fecundity and egg size in wild females

There were no significant differences in the length of the abdomen between the captured morphs (andromorph, 27.73 ± 0.15 mm (n = 26); gynomorph, 27.60 ± 0.15 mm (n = 34), t = 0.497, P = 0.619), or in the length of the hind wing (andromorph, 20.03 ± 0.14 mm; gynomorph, 19.94 ± 0.16 mm, t = 0.066, P = 0.947). The number of ovarioles was significantly higher in andromorphs (304.1 ± 13.4) than in gynomorphs (266.8 ± 10.2) (z = –8.512, P < 0.001 (Fig. 1A). The number of immature eggs in each ovariole did not differ between the morphs (andromorph, 25.8 ± 0.9; gynomorph, 24.6 ± 0.9) (z = –0.632, P = 0.527) (Fig. 1B). Consequently, the total number of immature eggs was greater in andromorphs (7925.6 ± 589.1, n = 16) than in gynomorphs (7164.1 ± 499.5, n = 14) (z = –23.91, P < 0.001).

The length of mature egg in gynomorphs was significantly larger than that of andromorphs, though the width of mature eggs did not significantly differ between the morphs (Fig. 1C, D).

Fecundity in laboratory reared females

Newly emerged individuals reared in the laboratory completely extended their wings within a couple of hours after eclosion and began to show flight activity. The lengths of the abdomen (andromorph, 25.69 ± 0.08 mm, n = 216; gynomorph, 25.63 ± 0.08 mm, n = 151) and hind wing (andromorph, 17.86 ± 0.06 mm, n = 216); gynomorph, 17.85 ± 0.06 mm, n = 151) did not differ between the morphs (abdomen, t = 0.497, P = 0.619; hind wing, t = 0.066, P = 0.947), though they were smaller than in the wild females.

Little egg development occurred for either morph within the first 3 days after emergence, during which time no mature or sub-mature eggs were found in the ovarioles. Andromorphs contained significantly more ovarioles (239.9 ± 7.9) in their ovaries than gynomorphs (206.7 ± 7.3) (z = –5.666, P < 0.001) (Fig. 2A). The average number of immature eggs per ovariole was approximately 20 in each morph (z = –0.58, P = 0.562) (Fig. 2B). The total number of immature eggs was higher in andromorphs (4770.1 ± 282.6, n =
than in gynomorphs (4070.0 ± 173.3, n = 24) (z = –36.44, P < 0.001).

Egg production and egg size in laboratory-reared females

Four days after emergence, each ovariole in both morphs contained either a single white-colored sub-mature egg or a single yellowish mature egg, suggesting that the onset of egg development did not differ between the morphs (Fig. 3). Four-day-old females of both morphs contained approximately 20 mature eggs. The number of mature eggs increased with age, reaching a peak 8 days after emergence in both morphs. The widths and lengths of mature eggs showed that gynomorphs developed a significantly larger mature egg than andromorphs (Fig. 2C, D).

**DISCUSSION**

We found no difference in the onset of egg development between the morphs in *I. senegalensis*, suggesting that the morphs undergo similar sexual maturation after emergence. However, the egg size and the total number of immature eggs (i.e., fecundity) differed between the morphs in both wild and laboratory-reared females, assuming that no additional ovarioles or immature eggs developed during the adult lifespan.

The genetic basis of female polymorphism has been elucidated in several damselfly species, including *Ceriagrion tenellum* (Andrés and Cordero, 1999) and *I. elegans* (Sanchez-Guillem et al., 2005). In *I. senegalensis*, the andromorphs are homozygous for a recessive allele, and the gynomorphs are either heterozygous or homozygous for the dominant allele, at an autosomal locus, with expression restricted to females (Takahashi and Watanabe, unpublished), as is the case for *I. damula* (Johnson, 1964) and *I. demorsa* (Johnson, 1966).

In animals with genetically determined color polymorphism, linkage disequilibrium between the color locus and loci for other traits, or pleiotropic effects of the color locus, often result in differences in morphology, physiology, behavior, and life-history strategy between the morphs (Forsman et al., 2008). The correlation between morph phenotype and reproductive traits in *I. senegalensis* might also result from linkage disequilibrium and pleiotropy.

Because they have a greater number of ovarioles, andromorphs in *I. senegalensis* might be able to produce a larger clutch than gynomorphs. The number of mature eggs developed tended to be higher in andromorphs than in gynomorphs in the laboratory-reared females. Because no significant difference in abdomen size between the morphs suggested that every female had a similar physical space for mature eggs, the smaller mature egg size in andromorphs than in gynomorphs is reasonable on the basis of a trade-off between egg size and number.

Female color polymorphisms correlated with reproductive strategies have been reported in a variety of taxa, including the owl *Strix aluco* (Roulin et al., 2003) and the lizard *Lacerta vivipara* (Vercken et al., 2006). In *I. senegalensis*, the andromorph and gynomorph have adopted *r*-strategic reproduction (high fecundity with small eggs) and *K*-strategic reproduction (low fecundity with large eggs), respectively. The andromorph might have the potential to lay more eggs than the gynomorph, assuming a sim-
lilar length of reproductive lifespan between the morphs, as in the case for other ischnurid damselflies (e.g., Andrés and Cordero, 2001). Sinervo et al. (2000) pointed out that population density differentially affects the fitness of r- and K-strategic morphs. Forbes et al. (1995) reported that the morph frequencies change with population density in the damselfly Nehalennia irene. Recent molecular evidence (Andrés et al., 2000) and empirical studies (Svensson et al., 2005) have also suggested that the female polymorphism in damselflies is under density-dependent selection.

We found that the size of mature eggs differed between the morphs, and that gynomorphs lay larger eggs than andromorphs. In Odonata, the size of early instar larvae depends on egg size (Schenk and Söndgerath, 2005). Egg developmental time also depends on egg size, due to the vitelline content (Hottenbacher and Koch, 2006). These observations suggest that the offspring of gynomorphs grow faster and form larger larvae than those of andromorphs. Because size-dependent cannibalism during the early larval stages is commonly observed in Odonata in aquatic environments, smaller larvae are apt to be eaten by larger ones (Anholt, 1994; Padefke and Suhling, 2003). Therefore, early instar larvae derived from gynomorphs must have higher survival than those from andromorphs.

In female polymorphic damselflies, it has been assumed that mating harassment is an important selection pressure for females, and that only their body color is subject to selection (Abbott and Svensson, 2008). Because the common morph in the population is more frequently harassed by males, negative frequency-dependent selection is a major force maintaining the female polymorphism (Svensson et al., 2005). We found differences between the morphs in reproductive traits, including fecundity and egg size, which determine the quantity and quality of the offspring, respectively, indicating that morph-specific reproductive traits would contribute to the fitness of each female morph, in addition to the negative effects of male harassment. Therefore, the differences in reproductive traits might potentially affect the overall fitness of each female morph, and thus be key factors affecting morph frequency in a population.

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