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COLOUR VARIATION IN FEMALE *LESTES DISJUNCTUS* SELYS: A SECOND EXAMPLE OF A POLYMORPHIC LESTID (ZYGOPTERA: LESTIDAE)

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Coexistence of discrete ♀ colour morphs is a common characteristic of many odon. species. Surveys have found that for some North American and European genera, half or more of the spp. show female-limited polymorphism, while in other genera, ♀ polymorphism appears far less common among spp. One such genus is *Lestes* with reportedly only one sp. (*L. sponsa*) being polymorphic. Here are described andromorphs and heteromorphs for *L. disjunctus*. Female-limited polymorphism might be more common, even in this genus, than is perceived currently. ♀ morph frequencies were estimated for 4 consecutive yr.: andromorphs constitute approximately 16% of mature ♀ ♀ sampled and this proportion is fairly consistent between years. Similar to other published reports on other spp., andromorphs and heteromorphs in this study population did not differ in wing length or mass. Seasonal patterns in representation of different morphs suggest that further research should be done on timing of emergence of andromorphs versus heteromorphs in this and perhaps other spp.

INTRODUCTION

Many species of damselflies and dragonflies exhibit discrete female colour morphs (e.g. FINCKE, 2004). Most typically, two (or sometimes three) mature female colour morphs are found in coexistence. One female morph (further referred to as heteromorph) is distinctively coloured compared to the male, while the other morph (andromorph) shows similar coloration and sometimes pattern

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as the conspecific male.

Laboratory crossing experiments have been completed for five species and indicate genetic inheritance of female polymorphism (e.g. ANDRÉS & CORDERO, 1999; SÁNCHEZ-GUILLÉN et al., 2005). Morph type appears determined by Mendelian genetics, with a number of alleles equal to the number of female colour morphs and alleles are likely situated on a single autosomal locus. Further, random genetic processes (e.g. genetic drift, founder effects) are insufficient to explain maintenance of female morphs (i.e. female-limited polymorphism is under selection; ANDRÉS et al., 2000, 2002; WONG et al., 2003).

Female morph differences are not restricted to variation in body coloration or pattern. Indeed, several studies have demonstrated differences in behavioural repertoires between andro- and heteromorphs (e.g. ROBERTSON, 1985; FORBES et al., 1997). For example, heteromorphs often occupy dense vegetation and avoid unwanted copulations by flying away from an approaching male, whereas andromorphs use more open habitat, do not fly large distances and directly face males in apparent agonistic interactions (e.g. FORBES et al., 1997; VAN GOSSUM et al., 2001). Several researchers have not found differences in body size measures between female morphs (CORDERO, 1992; THOMPSON, 1989; FORBES, 1994; LAJEUNESSE & FORBES, 2003). CORDERO et al. (1998), however, reported andromorphs were on average larger compared to heteromorphs in their study on *Ischnura elegans*, indicating the possibility that morphs for some species or some populations may differ in morphometrics.

In the majority of species where variation in female morph frequencies has been examined, the frequency of andromorphs appears relatively constant within a population (FORBES et al., 1995; CORDERO & EGIDO PÉREZ, 1998; SIROT, 1999). McKEE et al. (2005) reviewed all current literature, and concluded that variation in andromorph frequencies between populations is limited and has been reported mainly in the order of 10-30%, with some variation dependent on species. Additionally, WONG et al. (2003) showed considerable stability or homogeneity of morph ratios within a few sites across years. It has been suggested that selection contributes to the maintenance of similar morph frequencies across nearby populations (ANDRÉS et al., 2000). However, one notable exception is the damselfly *I. elegans*, for which a survey of morph frequencies in NW Spain showed inter-population variability in andromorph frequencies ranging from 4--91% (SÁNCHEZ-GUILLÉN et al., 2005).

Recently, FINCKE (2004) reviewed the distribution of female-limited polymorphism throughout suborder, family and genus for the North American and European species. Her overview indicates that such polymorphism is three times more common in genera of Zygoptera compared to Anisoptera. Second, in some species-rich damselfly genera, noteworthy *Ischnura*, *Enallagma* and *Argia*, about 50% or more of the North American and European species show female-limited polymorphism. FINCKE (2004) excluded two polymorphic species (*Lestes sponsa*

and *Libellula jesseana*) from her analysis because each represented less than 5% of otherwise monomorphic genera.

Here, we report on female-limited colour polymorphism in female *Lestes d. disjunctus* Selys (hereafter referred to as *L. disjunctus*). We describe the phenotype of the andromorph in comparison to the conspecific male and the heteromorph. We also provide information on the frequency of andromorphs in one population for four consecutive years, and examine whether andro- and heteromorphs differ in wing length and/or mass. The approximate age of heteromorphs and andromorphs was also assessed to determine whether this colour variation is indeed a typical adult colour polymorphism or simply an age effect with some older females acquiring pruinosity. Finally, we noted when andromorphs and heteromorphs were observed first during the season, in three years of study.

METHODS

Lestes disjunctus is a widely distributed species in Canada and is somewhat smaller than other *Lestes* species (WALKER, 1953). The species frequents permanent marshes or bogs and is one of nine *Lestes* species common to central Canada. At emergence, males and females appear similar in colour showing a bronze coloration with the pale areas being a dull yellowish colour (WALKER, 1953). *L. disjunctus* maturation occurs in just over 2 weeks after emergence with females taking on average 2 days longer than male conspecifics (SAWCHYN & GILLOT, 1974). It is at maturity that males develop a bluish pruinosity not previously described for females (WALKER, 1953).

All surveys and collections were completed at Barb's Marsh, located near the Queen's Biology Station (45°37'N, 76°13'W). As described by ANHOLT (1997), the marsh also has a seasonally flooded area and both marsh and flooded area are bounded by a hay field and mixed woods. We found *Lestes* species in all stages of maturity primarily in the seasonally flooded area as well as some individuals in the surrounding hay field. Thus, all surveys were concentrated in these areas. At Barb's Marsh *L. disjunctus* emergence usually begins at the end of June and adults can be observed as late as 1 August. Both *L. rectangularis* and *L. congener* also emerge from the marsh but are not as abundant (T. Robb, unpublished data).

Daily surveys, for adults of all *Lestes* species, which were one hour in duration (1100-1200h), were conducted in the surrounding area of the marsh in 2002, 2003 and 2005. Surveys were not completed on days when it rained between 1100-1200h. We began conducting the one-hour surveys on 29 June through to 1 August in 2002 (13 surveys in total), 28 June through to 19 July, 2003 (17 surveys in total) and 4 July through to 31 July, 2005 (21 surveys in total). The date of the initial survey corresponded to when the first young adult *L. disjunctus* was observed. During surveys, all *L. disjunctus* observed were netted. Upon capture, the sex, age of the adult (teneral, young or mature adult) was recorded, according to WALKER (1953). We also measured the distance between nodus and distal end of pterostigma of the right forewing for each individual (to the nearest 0.01 mm), using Mitutoyo® digital calipers. If an individual was female, we noted whether she displayed andromorph or heteromorph characteristics (see Result section for descriptions). Prior to release, each damselfly was given a mark on one of the hindwings to prevent recapture on that day. Marking was done with a permanent marker (Stanford® fine point Sharpie) and colour combinations were unique to date of marking. Recaptures on subsequent days were noted.

In 2004, we did not survey. However, we collected mature adult *L. disjunctus* between 11 July and 19 July. For all individuals collected, we recorded sex, length of the right forewing as above and also mass (to the nearest 0.001 g), using a Mettler® AE100 Digital Scale. For adult females, we recorded

if they had andromorph or heteromorph characters.

For each damselfly surveyed, we also counted the number of parasitic water mites (*Arrenurus pollictus*) and we scored engorgement for each mite. The parasitic mites that were found on *L. disjunctus* engorge throughout the host's maturation period and drop off their hosts during bouts of reproduction centred at appropriate water bodies or marshes (cf. SMITH, 1988). Thus the degree of engorgement can provide some indication of the age of the host following MITCHELL (1969) and SMITH & COOK (1991). In addition, mite scars indicate that engorged mites have dropped off recently suggesting an individual has had at least one bout of reproduction. The degree of engorgement was scored using a similar scale, previously used for a related mite species (FORBES *et al.*, 1999). Mite engorgement scores were 0, 1, 2, 3, 4 or 5 where 0 was given if the mite body was flat with no separation between ventral sclerites and the dorsal plate (no engorgement) and a score of 5 was given for fully engorged mites. Average mite engorgement scores for each damselfly were calculated.

Statistical analyses were completed in R (version 2.1.1, IHAKA & GENTLEMAN, 1996).

RESULTS

We observed two distinct *Lestes disjunctus* female morphs within only the mature individuals. For visual examination of the female morphs and males, digital images may be obtained upon request from mforbes@ccs.carleton.ca. Heteromorphs never showed blue coloration, and were lighter in appearance with a similar coloration to conspecific males prior to pruinosity (WALKER, 1953). Typically, andromorphs had several male-like characters in terms of coloration. Andromorphs had blue coloration on the rear of the head, pronotum, lower parts of the pleura of the pterothorax and on abdominal segments I, IX and partially on segment X. The greenish coloured antehumeral stripe of andromorphs also was similar to males, however, similar to heteromorphs, the mid-thoracic and humeral streak was broader than those observed on male conspecifics. WALKER (1953) previously described that only mature males showed the bluish pruinosity on specific areas of the body, most notably at the rear of the head, sterna and lower parts of the pleura of the pterothorax, and abdominal segments I, II, VIII, IX and X.

We report on numbers of female morphs for only mature individuals as we only observed andromorph characteristics on mature females in all surveys. During 2002 we observed a total of 228 mature individuals of which 69 were female and 159 were male. During 2003 we observed 231 mature individuals, of which 28 were female and 203 were male. Although we had more sampling days in 2005 we observed only 174 individuals of which 116 were female and 58 were male. Of the 133 mature individuals collected in 2004, there were 53 females and 80 males.

The first andromorph appeared in our surveys on 16 July in 2002 and 6 July in 2003 while mature heteromorphs and males were observed before those dates (Fig. 1). Andromorphs were observed on the first day of collection, 4 July, along with both mature heteromorphs and males during the 2005 surveys (Fig. 1). Andromorph frequencies were 15.9%, 17.9% and 17.2% for the surveys completed during the years 2002, 2003 and 2005, and for the collection in 2004 the andromorph fre-

quency was 13.2%. The frequency of andromorphs was not different between years ($\chi^2 = 0.79, df = 3, p = 0.85$).

There were very few recaptures over the course of the surveys in 2002 (2 recaptures) and in 2003 (4 recaptures) and it was only males that were recaptured in both those years. However we did recapture 36 individuals in 2005, of which 19 were heteromorphs and 7 were andromorphs. In support of our hypothesis heteromorphs recaptured between 1 and 18 days past initial capture date still exhibited heteromorph characters. We observed worn wings as well as some pruinosity on the ventral surface of the thorax on the individuals collected during the last two surveys of 2005 indicating the end of the flight season. We did not note worn wings during the 2002 and 2003 surveys.

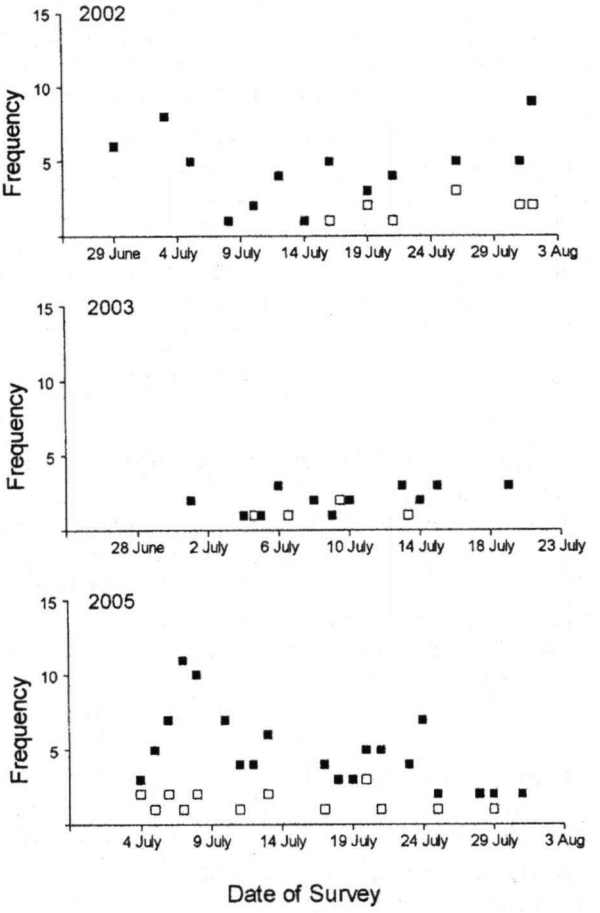

Fig. 1. Daily frequencies of andromorphs (open square) and heteromorphs (closed square) observed during the surveys conducted in 2002, 2003 and 2005. The initial date corresponds to the first day of the surveys for that particular year.

We report on mite engorgement and mite scars only for the year 2005 as this had the greatest number of days when both heteromorphs and andromorphs were observed with mites (Tab. I). The average mite engorgement scores did not differ between andromorphs and heteromorphs (Tab. I; $W = 171, p = 0.68$). Mite scars were observed on 6 of the 20 (30%) andromorphs observed with the first andromorph to have mite scars observed on the 8 July 2005. Of the 96 heteromorphs

Table I
Medians (interquartile ranges in brackets) of the average mite engorgement score on dates when both heteromorphs and andromorphs were observed during the 2005 survey. Only damselflies with mites were used in calculation of medians and for comparison

	Andromorph		Heteromorph	
Date	n	Median	n	Median
4 July	2	2.99 (2.98-3.00)	2	2.50 (2.00-3.00)
5 July	1	3.97	5	2.88 (2.25-3.50)
6 July	2	4.02 (4.00-4.03)	6	4.00 (2.82-4.02)
7 July	1	4.00	11	4.08 (4.00-4.20)
8 July	1	3.40	7	4.16 (4.00-4.71)
11 July	1	5.00	1	4.17
20 July	3	4.08 (4.00-4.42)	2	3.43 (3.00-3.86)

observed 16 (16.7%) had scars with the first heteromorph to have mite scars observed on the 11 July 2005.

There were no differences in size (wing length) between andromorphs and heteromorphs surveyed in 2002, 2003 and 2005 (Tab. II; 2002, $W = 394$, $p = 0.22$; 2003, $W = 77.5$, $p = 0.24$; 2005, $W = 1048$, $p = 0.85$). Neither were there differences in wing length or mass between andromorphs and heteromorphs collected in 2004 (Tab. II; wing length, $W = 196.5$, $p = 0.36$; mass, $W = 150$, $p = 0.78$).

DISCUSSION

Two distinct female types, one heteromorph and one andromorph, were found to occur for a *Lestes disjunctus* population in central Canada. Andromorphs were similar in coloration to male conspecifics, however pattern (both the mid-thoracic and humeral streak) was similar to heteromorphs. The frequency of andromorphs was similar between all years examined and ranged between 13.2% in 2004 to 17.9% in 2003. This range of frequencies and similarity between years is comparable to other odonate species exhibiting female-limited polymorphism (e.g. McKEE et al., 2005).

We found no body size differences between heteromorphs and andromorphs for all years nor did we find differences in body mass for the individuals collected in 2004. Although there are instances where female morphs differ in body size (CORDERO et al., 1998) there has been several studies indicating no differences in body size between female morphs (e.g. LAJEUNESSE & FORBES, 2003 and references therein).

Andromorphs were first observed mid- to late-season in 2002 and mid-season in 2003. While in 2005 andro- and heteromorphs were observed on the first day of the survey. An earlier study found that sexually mature *Enallagma boreale* andromorphs appeared in the population earlier in the season than did heteromorphs

Table II
Female andromorph and heteromorph median wing length (mm) and interquartile ranges (IQR) for individuals surveyed during 2002, 2003 and 2005. Median and IQR of wing length (mm) and mass (g) for female andromorphs and heteromorphs collected during 2004 are also presented (see text for statistical comparisons)

	Andromorph		Heteromorph	
	Median	IQR	Median	IQR
2002	13.85	13.40-14.26	14.20	13.90-14.76
2003	14.63	14.23-14.66	14.88	14.43-15.22
2005	14.63	13.96-14.98	14.60	14.17-14.98
2004				
- wing length	14.65	14.05-15.24	14.99	14.50-15.56
- mass	0.638	0.613-0.733	0.648	0.571-0.708

(FINCKE, 1994). In that study, FINCKE (1994) suggested that if the genes for body colour and timing of emergence were in linkage disequilibrium, then this linkage disequilibrium could explain andromorphs appearing earlier. If anything, it seems andromorphs are appearing at the same time or later than heteromorphs in *L. disjunctus*. It would be necessary, however, to obtain actual emergence dates for the different morphs to address FINCKE's (1994) idea, adequately.

This study provides a second species from the Lestid family to display female-limited polymorphism. It is unlikely that this is a case of age-related colour polymorphism (all females would acquire the male like colouration as they age) for several reasons. First both andro- and heteromorphs were found with similar mite engorgement scores throughout the flight period suggesting that andromorph characters develop early on in maturation and only for some individuals. We also found fully engorged parasitic mites on the thorax of both andromorphs and heteromorphs. Mite scars were observed on both heteromorphs and andromorphs beginning at similar times of the flight period. If blue pruinosity developed after reproductive maturation we would expect to find andromorphs only late in the season and with mite scars. During our 2005 survey we recaptured several females with heteromorph characters and none showed any sign of developing andromorph characters (one individual was caught 18 days after the initial capture date). We also did not observe an increase in the number of andromorphs over the course of the season. Taken together, these data suggests that andromorphs and heteromorphs are of similar age.

We have yet to address any behavioural differences between andromorphs and heteromorphs for this species. A more intense examination of other *Lestes* species and other odonate species may be necessary to appreciate better how common female-limited polymorphism is for damselflies and dragonflies. It is interesting to note that, although not reported, andromorphs of *L. disjunctus* were observed during a previous study on the same population (B. Anholt, pers. comm.). De-

spite earlier intensive work on many damselfly species, the presence of female-limited polymorphism whether genetic or age-related may have been observed, but not documented.

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