Heredity **64** (1990) 341-346

Received 4 October 1989

The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur) (Odonata: Coenagrionidae)

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The damselfly *Ischnura graellsii* has three female coloration patterns: the male-like coloured or androchromatypic (A) females, and two gynochromatypic females: *infuscans* (olive-green to brown females, I), and *aurantiaca* (orange to brown females, O). The inheritance of this polymorphism was studied by rearing the offspring of 33 laboratory crosses. Fourteen F_1, eight F_2 and eleven F_3 crosses produced more than 2400 adults in laboratory conditions. Results of these crosses indicate: (1) the progeny of one female can consist of one, two or all phenotypes; (2) when two phenotypes occur the ratio is 1:1 or 3:1; and (3) when all phenotypes occur the ratio is 2:1:1 for A:I:O females. The simplest hypothesis to explain these results is that three alleles of one autosomal locus control this polymorphism. The androchromatypic allele (p^a) is dominant over both gynochromatypic alleles, and *infuscans* (p^i) over *aurantiaca* (p^o) (Dominance: $p^a > p^i > p^o$). Males possess all six possible genotypes but only one phenotype (p^a). All matings were compatible with the hypothesis, and the presence of all genotypes was proved with the matings obtained. Hypotheses about the maintenance of female polymorphism in *Ischnura* damselflies so far studied are revised.

INTRODUCTION

Many damselfly species have polymorphic females, one morph resembles the male (which is monomorphic), the other females being more or less dissimilar. In this paper, I will call "androchromatypic" the females with male coloration pattern and "gynochromatypic" the others, using the terminology proposed by Hilton (1987). This kind of polymorphism is common in the family Coenagrionidae. European species of *Pyrrhosoma*, *Ceriagrion*, *Ischnura*, and *Enallagma* are particularly polymorphic, and most *Coenagrion* species show many female coloration patterns (d'Aguilar *et al.*, 1985; Askew, 1988), but there is no clear evidence whether they are age-related colour phases or genetically distinct female types. Female polymorphism has also been noted in Polythoridae (Bick and Bick, 1986), Calopterygidae (Dumont, 1972), Libellulidae (D. R. Paulson, personal communication to Robertson, 1985; Kumar, 1988) and Aeshnidae (Corbet, 1986). Female polymorphism is therefore a common phenomenon in the order Odonata.

Johnson (1964, 1966) made the first and only genetic studies of this polymorphism. He showed that in *Ischnura damula* and *I. demorsa* there are two female phenotypes, and that the inheritance may be explained by means one autosomical locus with sex-restricted expression, androchromatypic females being homozygous recessive (hh), and gynochromatypic females heterozygous (h^+h) and homozygous dominant (h^+h^+).

In *I. graellsii* there are three female phenotypes, which show different colour changes throughout sexual maturation (fig. 1) (Cordero, 1987). Androchromatypic females (A), are blue in mature phase, as well as the males. There are gynochromatypic females of the *aurantiaca* form (O), and of the *infuscans* form (I), both showing brown thorax in mature phase, but different young phases (fig. 1). It is possible to differentiate the female phenotypes in all colour phases by means of the extension of antehumeral stripes of thorax and a black spot on the dorsum of the eighth abdominal segment (Cordero, 1987).

This polymorphism could be explained by an hypothesis similar to that described for *I. damula* and *I. demorsa*, but with codominance relationships between the two alleles of the locus "p" (polymorphism), A females being homozygous pp, O females homozygous p^+p^+ and I females heterozygous p^+p (Cordero, 1987). The same hypothesis was proposed by Hinnekint (1987) for

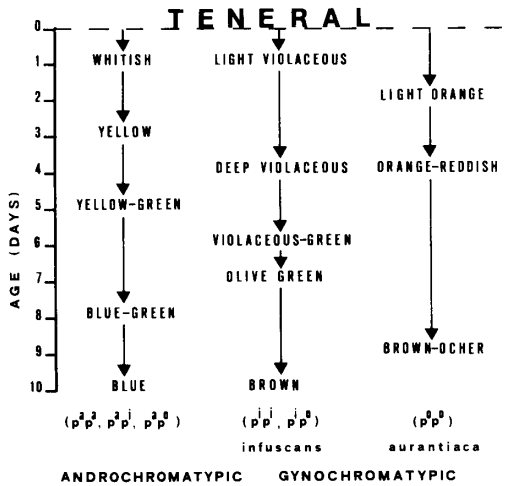

Figure 1 The relationship between thoracic colour changes and age in the different phenotypes of *I. graellsii*. Proposed genotypes are shown in parenthesis. Teneral are newly emerged individuals. Sexual maturation is obtained after 5–6 days.

I. elegans, which also has three female phenotypes.

In this paper I present the results of controlled laboratory matings in order to test this hypothesis, and review how female polymorphism could be maintained in natural populations of *Ischnura* damselflies.

METHODS

The scarcity of genetic studies with dragonflies may be explained by the difficulty of obtaining laboratory matings and the rearing of a great number of larvae in small spaces. This study was made with adults of *I. graellsii* (a small damselfly common in the Iberian Peninsula) obtained from different populations of Pontevedra (NW Spain).

Five insectaries of $50 \times 50 \times 50$ (or 70) cm with numerous wood switchs as perching substrates were used to obtain matings. Insectaries were covered with aluminium foil, which reflects sufficient light and impedes escape responses (Johnson, 1965), and several culture bottles of *Drosophila* were added to supply food. Insectaries were placed in a chamber maintained at 21–23°C, 60–80 per cent humidity and a photoperiod of 15L:9D hours. In these conditions matings of *I. graellsii* were easy to obtain. Maximum longevity was 40 days, longer than the one month observed in the field (Cordero, 1987). In the insectaries I was also able to elicit mating of *I. elegans*, *I. pumilio*, *Ceriagrion tenellum*, *Lestes viridis*, *L. virens*, *L. barbarus* and *L. dryas*.

Teneral specimens (1–2 day old adults) were captured in the field and transported to the laboratory for maturing, in order to obtain virgin females to begin the experiments. Each specimen was individually marked with a number on its wings. Mature females were introduced into insectaries of males to mate, and each pair was observed continuously or isolated until copulation was completed. Mated females were placed every 2–3 days in individual oviposition chambers, with damp filter paper as an oviposition substrate. Eggs were maintained in Petri dishes in the same conditions as adults.

After egg eclosion, small larvae were maintained in plastic boxes filled with water, with filter paper as perching substrate, and nauplius of *Artemia salina* as food (Miguel Conesa, personal communication). Medium-sized larvae (>6 mm) were placed in individual plastic tubes, to avoid cannibalism. This method is time-consuming because of the need to change the water and add food at least every second day. Large larvae (>1 cm) were placed in separate cells, but in the same water body, which allowed reducing the frequency of water changes to once each week and standardized breeding conditions. These larvae were fed with *Cloeon* larvae (Ephemeroptera) and aquatic oligochaeta (*Lumbriculus variegatus*) until emergence. Last instar larvae were maintained in plastic containers with numerous wood switches as emergence supports.

More than 2400 adults were obtained with this method, from August 1987 to July 1989, with one generation interval of approximately 5 months. All females were assigned to each coloration type in the first hours after emergence, and some were maintained until maturation. All showed the coloration change of their colour type. In the F_1 all larvae were reared, but in the F_2, to increase sample size, most male larvae were rejected. in the F_3, larvae were reared in plastic boxes, without separation during the early instars, because I was only interested in obtaining homozygous lines. For this reason, sample sizes were smaller.

Observed and expected values were compared using χ^2 tests.

RESULTS AND DISCUSSION

Results of laboratory matings

The segregation of female phenotypes in F_1 crosses of *I. graellsii* is shown in table 1. Results obtained

Table 1 Percentages of the different female phenotypes in the F_1 generation of *Ischnura graellsii*. Expected frequencies after the hypothesis of one locus and three alleles are shown in parenthesis (see the text). N = number of females

Cross code	Percentage of males*	N	Female phenotype A	I	O	χ^2	p
Androchromatypic							
♀H-♂1	49·1	54	48·1 (50)	51·9 (50)	0·0 (0)	0·07	0·786
♀R-♂2	50·5	47	89·4 (75)	10·6 (25)	0·0 (0)	5·17	0·023
♀S-♂3	44·3	59	76·3 (75)	23·7 (25)	0·0 (0)	0·05	0·822
♀T-♂2	48·9	44	72·7 (75)	27·3 (25)	0·0 (0)	0·12	0·728
♀B-♂4	54·8	14	100·0 (100)	0·0 (0)	0·0 (0)	—	—
♀N-♂5	45·0	11	45·5 (50)	36·4 (25)	18·2 (25)	0·82	0·664
infuscans							
♀C-♂6	55·9	30	0·0 (0)	100·0 (100)	0·0 (0)	—	—
♀J-♂4	41·3	27	0·0 (0)	100·0 (100)	0·0 (0)	—	—
♀K-♂7	41·7	28	0·0 (0)	100·0 (100)	0·0 (0)	—	—
♀L-♂8	41·7	32	0·0 (0)	53·1 (50)	46·9 (50)	0·13	0·724
aurantiaca							
♀M-♂9	50·0	44	0·0 (0)	40·9 (50)	59·1 (50)	1·45	0·228
♀P-♂5	54·4	46	0·0 (0)	43·5 (50)	56·5 (50)	0·78	0·376
♀U-♂3	49·0	51	49·0 (50)	0·0 (0)	51·0 (50)	0·02	0·889
♀X-♂2	50·5	49	40·8 (50)	59·2 (50)	0·0 (0)	1·65	0·199

* Obtained from adults.

indicate that: (1) a single female may produce progeny of one, two or three female phenotypes; (2) if two phenotypes are present, the ratio is 1:1 or 3:1; and (3) when all phenotypes are present the ratio is 2:1:1 for A:I:O females. These results reject the hypothesis of one autosomal locus with two alleles (Cordero, 1987), because if A and O females are homozygous for different alleles, they cannot be present in the same progeny from a single-pair mating.

The simplest hypothesis that explains the observed F_1 segregation, postulates the existence of one autosomal locus with three alleles, p^a, p^i and p^o, with dominance relationships: $p^a > p^i > p^o$. On this hypothesis, A females may have three possible genotypes: p^ap^a, p^ap^i and p^ap^o; I females two genotypes: p^ip^i and p^ip^o; and O females one genotype: p^op^o. Males could have all genotypes, but only one phenotype. Almost all crosses agreed with this hypothesis. The progeny of female R (table 1) had more A females than expected ($P = 0{\cdot}023$), nevertheless, when both crosses of male 2 with A females are considered jointly, there are no differences from expected ($P = 0{\cdot}164$).

Males crossed with different females (males 2, 3, 4 and 5) showed one genotype consistent with all crosses. The progeny of female N are very interesting, because all phenotypes are present, indicating a cross with two heterozygotes for different alleles: male 5 is judged to be p^ip^o (as indicated by his cross with female P) and female N to be p^ap^o (because she is an A female with O daughters).

To test this hypothesis further, I made 8 F_2 crosses, results of which are shown in table 2. All crosses were consistent with the hypothesis and with the genotypes assigned to F_1 individuals. This generation yielded a second mating with all female phenotypes in the progeny: the cross between female N1, whose genotype deduced from the F_1 results should be p^ip^o, and male X1, with genotype p^ap^o (indicated by the segregation in his matings with the three female penotypes).

A third generation was reared in order to obtain some homozygous lines. Results of this F_3 are shown in table 3. All crosses agreed with expected following the genotypes assigned to each individual, excepting the progeny of female H15, which yielded a ratio 1 A:1 I while the expected was 3 A:1 I. This cross gave only 18 females, and I assume that this segregation reflects sampling error.

An important result of this work is that androchromatypic allele is dominant in *I. graellsii*, while in *I. damula* and *I. demorsa* it is recessive (Johnson, 1964, 1966). The biological significance of this fact is not evident.

Taking into account all matings obtained, the existence of all genotypes proposed by the hypothesis was demonstrated, as the analysis of tables 1-3 reveals. The sex-ratio (tables 1-3) is 1:1 excepting the cross of female U1, with only 40 per

Table 2 Percentages of the different female phenotypes in the F_2 generation of *I. graellsii*. Expected frequences are in parenthesis. The letter which identifies each individual is the code of the parental female of the F_1. N = number of females

Cross code	Percentage of males*	N	Female phenotype A	I	O	χ^2	p
Androchromatypic							
♀U1-♂M1	40·0	69	50·7 (50)	0·0 (0)	49·3 (50)	0·01	0·904
♀S1-♂X1	45·7	57	100·0 (100)	0·0 (0)	0·0 (0)	—	—
♀H1-♂S2	56·0	67	100·0 (100)	0·0 (0)	0·0 (0)	—	—
infuscans							
♀S3-♂C1	55·1	71	0·0 (0)	78·9 (75)	21·1 (25)	0·57	0·451
♀C2-♂C1	52·4	65	0·0 (0)	100·0 (100)	0·0 (0)	—	—
♀N1-♂X1	51·5	64	54·7 (50)	26·6 (25)	18·8 (25)	1·34	0·511
aurantiaca							
♀*M2*-♂*M3*	55·9	50	0·0 (0)	46·0 (50)	54·0 (50)	0·32	0·572
♀P1-♂X1	48·4	62	46·8 (50)	0·0 (0)	53·2 (50)	0·26	0·612

* Obtained from larvae.

Table 3 Percentages of the different female phenotypes in the F_3 generation of *I. graellsii*. Expected frequences are in parenthesis. First letter and number which identifies each individual is the code of the parental female of the F_2. N = number of females

Cross code	Percentage of males*	N	Female phenotype A	I	O	χ^2	p
Androchromatypic							
♀H11-♂H12	40·7	32	100·0 (100)	0·0 (0)	0·0 (0)	—	—
♀H13-♂H14	41·7	21	100·0 (100)	0·0 (0)	0·0 (0)	—	—
♀H15-♂S11	62·5	18	50·0 (75)	50·0 (25)	0·0 (0)	6·00	0·014
♀H16-♂H17	46·9	26	84·6 (75)	15·4 (25)	0·0 (0)	1·28	0·258
infuscans							
♀C21-♂C22	46·9	51	0·0 (0)	100·0 (100)	0·0 (0)	—	—
aurantiaca							
♀U11-♂M21	31·6	26	0·0 (0)	46·2 (50)	53·8 (50)	0·15	0·695
♀P11-♂M22	49·2	30	0·0 (0)	40·0 (50)	60·0 (50)	1·20	0·273
♀P12-♂P13	38·5	40	47·5 (50)	0·0 (0)	52·5 (50)	0·10	0·752
♀S31-♂P14	62·1	22	50·0 (50)	0·0 (0)	50·0 (50)	—	—
♀P15-♂U12	64·3	15	0·0 (0)	0·0 (0)	100·0 (100)	—	—
♀U13-♂P16	41·7	28	0·0 (0)	0·0 (0)	100·0 (100)	—	—

* Obtained from adults.

cent males ($P = 0·020$), and the cross of female U11, with 32 per cent males ($P = 0·023$). Therefore female biased sex-ratios found by Johnson (1964, 1966) were not observed.

Maintenance of polymorphism

Androchromatypic females of *I. graellsii* mimic male coloration and behaviour, and males are not able to distinguish these females from other males (Cordero, 1989), as occurs in *I. ramburi* too (Robertson, 1985). In the insectaries, androchromatypic females refuse a second mating in the same day, which is common in gynochromatypic females (Cordero, 1989). As suggested by Robertson (1985), this difference may have an adaptive significance if it is taken into account that copulations in this species may exceed 5 hours (time during which females are unable to eat and are perhaps more exposed to predation) and that once-mated females are able to fertilize all eggs laid throughout their lifespan (Cordero, in press). Androchromatypic females may be favoured by high density conditions, when the encounters with males are very common. For Hinnekint (1987) this fact and the existence of pluriannual cycles with

Table 4 Percentages of the different female phenotypes in five natural populations of *I. graellsii* of Galicia (NW Spain). All samples in the same generation interval are considered jointly

Population	Date	N	Female phenotype (percentage)		
			A	I	O
Artificial pond, Salcedo, Pontevedra	Jul-Aug 1985	523	18·0	69·2	12·8
Salt marsh, Lourizán Pontevedra	Aug-Sep 1986	29	20·7	75·9	3·5
	May 1987	65	16·9	76·9	6·2
	Aug 1987	685	13·6	75·6	10·8
	May-Jun 1988	85	8·2	78·8	12·9
	Sep 1988	107	17·8	71·0	11·2
Artificial pond, O. Rosal, Pontevedra	Aug 1986	46	6·5	78·3	15·2
Artificial pond, Corrubedo, A Coruña	Aug-Sep 1988	94	18·1	70·2	11·7
	Jul-Aug 1989	97	11·3	78·4	10·3
Lagoon, Vilar, A Coruña	Jul 1989	37	29·7	64·9	5·4

variation in population density, explain the maintenance of female polymorphism in *I. elegans.*

In an alternative explanation proposed by Johnson (1975) for *I. damula* and *I. demorsa*, androchromatypic females may offer increased reproductive isolation between the species in sympatry, but are perhaps more vulnerable to visual predators, as a result of their conspicuous coloration. Gynochromatypic females engage in heterospecific matings when the species are sympatric, thereby lowering their reproductive potentials (Johnson, 1975).

The relative advantage of androchromatypic females probably differs for each species and in different ecological conditions for the same species. Robertson (personal communication) in a comparative study of the thirteen North American species of *Ischnura* found extremes in androchromatypic frequencies, resemblance to males, copulation duration and frequency, and postcopulatory guarding. This comparative study generally supports the hypothesis that androchromatypic frequencies are related to the mating behaviour of each species. Thompson (1989) was unable to find any difference between andro- and gynochromatypic females of *Coenagrion puella* in date of maturation, size, mature lifespan, and number of clutches of eggs laid. Furthermore there is no supporting evidence for higher predation risks for andro- than gynochromatypic females of any species.

Gynochromatypic *infuscans* females are the majority of *I. graellsii* females: 65–79 per cent in five sampled populations, androchromatypic (7–30 per cent) and *aurantiaca* (4–15 per cent) are less common (table 4). Preliminary data suggest that androchromatypic females are more frequent in dense populations, but this is difficult to test because *I. graellsii* is very rarely seen forming low density populations (the only low density population sampled was at an artificial pond at O Rosal, Pontevedra, table 4). Furthermore, as table 4 shows, the frequency of androchromatypic females varies between 8 and 21 per cent in the same population at different dates. If males have genetically determined preference to mate with one type of female, as reported in *I. damula* and *I. demorsa* (Johnson, 1975), then natural selection can be more intense, but this preference does not exist in *I. ramburi* (Robertson, 1985) or in *I. graellsii* (Cordero, unpublished data). Finally, the existence of fitness differences between andro- and gynochromatypic larvae is unknown, although the larva is the longest phase of most odonates.

To explain the maintenance of female polymorphism of *I. graellsii* the study of the reproductive strategies of different types of females in different ecological conditions is needed.

Acknowledgements I wish to thank Hugh H. Robertson, Gonzalo Alvarez and Emilio Valadé for their comments for the manuscript. Enrique Martínez-Ansemil identified the *Lumbriculus.* This paper was presented to the "X International Symposium of Odonatology", Johnson City, Tennessee, USA, in August 1989. This study was aided by a fellowship from the Spanish Ministry of Education and Science to the author (Plan de Formación del Profesorado y del Personal Investigador).

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