

POLYMORPHISM AND NATURAL SELECTION IN ISCHNURAN DAMSELFLIES

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ABSTRACT:

The data for sex-limited female dimorphism and its likely interaction with natural selection are summarized. The male-like andromorphs offer increased reproductive isolation, but are more vulnerable to predation. The cryptic heteromorphs have higher resistance against predation but engage in interspecific mating, thereby lowering their reproductive potentials. These observations appear to explain the higher observed frequencies of andromorphs in sympatric assemblages. Male mating preferences exist, with androphilic and heterophilic types having a genetic determination similar to that of the female morphs. The system allows a rapid restructuring of the populations with shifts of sympatry, though additional associations with fitness must exist.

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Introduction

This study summarizes the available data on sex-limited polymorphism in Ischnura damula and I. demorsa relative to its role in natural selection. Most ischnuran damselflies exhibit sex-limited, female dimorphism consisting of two color pattern morphs. One morph is similar to its conspecific male, the andromorph, and this combination of color and pattern is distinct between ischnuran species. Judging from man's ability to perceive optical stimuli, the andromorphic patterns, often associated with a metallic reflection, deprive individuals of a camouflaged attribute in normal environments. The second female form, the heteromorph, is a drab combination of tan and dark brown, well-concealed in typical habitats, and appears quite similar between ischnuran species. Morph patterns and inheritance in I. damula and I. demorsa have been reported (Johnson, 1964a; 1966a) providing the following data. The morphs of I. damula are distinct throughout adult life while females of I. demorsa develop, two to three days after emergence, a pruinescence on the outer body cuticle. This exudate reduces morph distinction on initial observation and all mating appears to occur prior to its appearance. Breeding tests have identified the dimorphism, in both species, with a single autosomal locus having two alleles with dominance, h^+ and h . Heteromorphic females include both h^+h^+ and h^+h genotypes while andromorphic females are homozygous recessive genotypes, hh . Males include all three genotypes but possess only the species-specific andromorphic phenotype. Morph frequencies vary between populations but are otherwise stable and show no seasonal change or association with any known physical or chemical features of the habitat.

Population Structure and Isolation Breakdown

Populations contributing to this study occur in southwestern New Mexico and range from cool mountain streams to the warm waters of the Rio Grande valley. Colonies occupying the Rio Grande valley with its warm, long growth season have 2 to 3 generations yearly (Johnson, 1964b). Single generations per year characterize the mountain habitats. Ischnura damula prefers standing water communities while I. demorsa favors streams and springs (Johnson, 1966b). This habitat

selection is subject to environmental conditioning in last instar larvae and does not ecologically isolate adults when both community types occur in close approximation. The initial spring emergence contributes more adult numbers than appear during any similar period of the flight season; however, emergence is continuous for most of the season. Populations consist therefore of heterogeneous age groups and generations tend to overlap where the species is multi-voltine. While not estimated in absolute numbers, population densities appear well above levels likely to have much effect by genetic drift.

Data are available on morph frequencies in the following 14 habitats:

1. Cook's Spring, 1 mile west of Socorro, N. M.
2. Escondida Park, 4 miles north of Socorro, N. M.
3. Bosque Del Apache National Wildlife Refuge, 16 miles south of Socorro, N. M.
4. Elephant Butte Dam, Elephant Butte, N. M.
5. Mangus Spring, 16 miles west of Aragon, N. M.
6. Tularosa River, 5 miles north of Aragon, N. M.
7. Allen Hoague Lake, 2 miles north of the Willow Creek Ranger Station, Gila National Forest, N. M.
8. Iron Creek, 10 miles northeast of the Willow Creek Ranger Station, Gila National Forest, N. M.
9. Whitewater Creek, Glenwood, N. M.
10. San Francisco River, Reserve, N. M.
11. Gila River, Cliff, N. M.
12. Bear Trap Canyon, 16 miles southwest of Magdalena, N. M.
13. Torreón Spring, 25 miles southwest of Socorro, N. M.
14. Ojo Caliente Spring, 30 miles southwest of Magdalena, N. M.

The first six sites were sampled weekly or biweekly during the flight seasons of five years, 1962 through 1967, with samples ranging from 30 to 72 individuals per species. Morph frequencies at each site were quite stable, the greatest range being 8.5 per cent for I. demorsa at Mangus Spring. The only feature having an apparent correlation with morph frequencies is the presence or absence of sympatric assemblage for the two species. These data are given in part 1 of Table I. The frequencies of andromorphic females are clearly higher for both species when in sympatry. Data for eight populations sampled in an irregular sequence over the study are given in part 2 of Table I. Where these populations are represented by two or more samples, the stability of frequencies was further observed and the association of high andromorphic frequencies with sympatry repeated. Andromorphic proportions represent homozygous recessives; however, the Hardy-Weinberg expression is not suitable for estimating gene frequencies as the samples have surely experienced some selection by the time of collection and mating seems not to be random as mentioned below. Thus the square root of these frequencies involve an unknown error relative to a gene frequency estimate. In allopatric conditions, andromorphic genotype frequencies of I. damula range from 2.0 to 6.5 per cent and for I. demorsa, the range is also 2.0 to 6.0 per cent. In sympatry, andromorphic genotypes range from 9.2 to 26.5 per cent for I. damula and similarly for I. demorsa, the range is 20.5 to 41.5 per cent.

Early stages of the population surveys revealed interspecific pairing. Heteromorphic females of I. damula were found in tandem with I. demorsa males and similar mispairing was found involving heteromorphic I. demorsa females. During the five-year study, 18 of 220 I. damula males collected in tandem held heteromorphic I. demorsa females, or 8.2 per cent. Seventeen of 140 I. demorsa collected in a similar fashion held heteromorphic I. damula females, or 12.1 per cent. Finding tandem pairs for I. demorsa is more difficult. Females of I. demorsa

of Fisher Trellis Diagrams. The daily sample of insects are given in the vertical captures column corresponding to the vertical date column. The number of captures marked and released on a given day are given by the figure at the far right of each row. Specimens damaged in collecting or marking were not released as their survival was obviously affected. Recaptures included in each day's sample are given in the body of the tables corresponding to the time of original capture relative to the bottom row of dates. For example, in Table 3 on July 7, 23 females were collected, marked or scored, and released. These females included four recaptures, three marked on July 5 and one marked on July 6. These recaptures yield seven observed days for the July 7 sample etc. Results of this analysis are given in Table 5 indicating the best fitting daily survival rates to be 0.90 and 0.74 for heteromorphic and andromorphic I. damula females respectively. Assuming survival constant over this period of time, these

Table 5. Observed and expected survival days for I. damula based on daily survival rates giving minimum net differences in observed less expected values assuming constant survival. Survival rates meeting this condition are given for each morph in parenthesis.

DATE	HETEROMORPHIC FEMALES (0.90)			ANDROMORPHIC FEMALES (0.74)		
	Observed Days	Expected Days	Observed less Expected	Observed Days	Expected Days	Observed less Expected
JULY 6	4	4	0	3	3	0
7	7	6.29	+0.71	7	7.51	-0.51
8	8	10.09	-2.09	9	9.79	-0.79
9	16	21.03	-5.03	5	6.58	-1.58
10	11	8.83	+2.17	13	16.11	-3.11
11	19	16.37	+2.63	12	9.09	+2.91
12	31	28.85	+2.15	13	11.73	+1.27
13	28	32.21	-4.21	20	19.10	+0.90
14	31	34.96	-3.96	10	9.86	+0.14
			-8.62			-0.77

rates correspond to mean life expectancies of 10.0 and 3.8 days respectively (Fisher and Ford, 1947). Both morphs of I. damula have been maintained in laboratory stocks for periods up to fifteen days and general experience with the laboratory colony suggests no differential morph survival. A satisfactory estimate of confidence intervals for the field estimates of survival is not available; however, the estimate for heteromorphic females consists of a life expectancy 62 per cent greater than for andromorphic females. If any bias in collecting occurred, the tendency would have been to miss the heteromorphs since they are clearly more cryptically adjusted to the habitat. A similar study of survival for I. demorsa morphs is not available and the following explanation assumes survival is similar to I. damula. However, since the period of distinct morph expression is less, due to the appearance of pruinescence, the selection effect may also be less.

The Dimorphism's Role in Natural Selection

Data presented above suggest the following explanation for the dimorphism's

role in natural selection. The pattern of mismating in sympatry allows partial breakdown in reproductive isolation reducing reproductive potentials of heteromorphic females. The longevity of heteromorphic females is however greater than found for andromorphic females probably due to visually-oriented predation. Allopatric conditions may be expected therefore to confer a net advantage to heteromorphic phenotypes. On exposure to sympatry, such predator-resistant advantage would be offset by lower reproductive potentials. These opposing forces of selection could produce the association of morph frequencies reported above relative to degree of interspecific exposure. I have not observed a population during an interval when sympatry was changing, but such an event should not be overly difficult to document where habitats are numerous and precipitation pattern varies. I furthermore predict that the change will be rapid, involving only a few generations.

The environmental change between lotic and lentic conditions often involves short intervals and occurs in an unpredictable fashion as precipitation cycles vary. Excepting extremely isolated habitats, these small insects can probably colonize suitable new sites rather quickly. The most efficient adaptation would reasonably reduce to a minimum the time exposed to undue selective disadvantage or consist of a rapid restructuring of the damselfly population. The loss of fitness in heteromorphs, by the breakdown of isolation during sympatry, may assume a large magnitude. Likewise, if the above estimates of morph survival are even approximately valid, a high selection coefficient may exist against andromorphs countered mainly by the improved reproductive isolation they offer. Consequently, large fitness differences could accompany a population's shifting in and out of sympatry. A rapid change of morph frequencies would predictably accompany these changes in fitness. The cost in numbers lost to selection would also have to be paid over a relatively short period. These insects probably average over 500 eggs per individual female and perhaps could bear the price. Species with lower reproductive output per individual may not be able to afford a rapid restructuring of their populations. Polygenes scattered over several chromosomes would not have the likelihood of fast frequency change possessed by traits governed through a single gene locus. A simple mode of inheritance is predictable in view of the suggested function recognizing that the *h* locus may in fact be a supergene. Secondly, the change in frequency of selection targets may also be accelerated by positive phenotypic assortative mating as a higher mean frequency of parent-like phenotypes results among individuals of separate broods. Evidence for two male mating-types has been obtained in both species. In ischnuran populations, such mating requires that males, all phenotypically andromorphic, discriminate between the two female morphs in mate selection. Such male discrimination appears likely only if genetically determined and the data also point to this conclusion.

Innate Basis for Male Mating Preferences

Inheritance studies of female dimorphism cited above incorporated an F_1 analysis with multiple male matings. The laboratory environment released mating behavior in most males; however, certain males consistently refused mating attempts with certain females and this difficulty was most evident for matings involving one male and both morphs. A study, using Krieger and Krieger-Loibl's (1958) techniques with *I. damula* and *I. demorsa*, screened for behavioral preferences of morphs within each species with the aid of individually marked males. Two male mating-types were recognized early in the project, one preferring andromorphic females, the androphilic male, and the other type discriminating in favor of the

heteromorphic females, the heterophilic male. This male discrimination was broken only by allowing a male's sex drive to reach peak activity by an absence from females for four to five days. A male isolated from its preferred female type can therefore be induced to mate with the alternate female; otherwise, once a male's preference is scored, it remains the same through the testing interval, three days for most individuals.

The sequence of reproductive behavior, for the species discussed here, appears similar to the activities Krieger and Krieger-Loibl (1958) report for other ischnurans. Procedures for scoring male preference were as follows. A series of five successive tests on each male sometime during the three day period following maturation was adopted. Males are sexually mature one day after emergence. A test consists of placing the male in a cage with an equal number of both mature female morphs. Female numbers ranged from three to six of each morph and adding more females did not seem to speed up the male's reaction. The male, having been caged either alone or with other males, then attempts tandem with a female, usually within twenty minutes. As described elsewhere, obtaining an actual cross may require several approaches before a female becomes receptive; however, the question here concerned only the male's choice. The male's first attempt at tandem was scored and he was later removed from the females until tested again. If males were exposed every two days in this fashion to both female types, their sex drive seems not to reach peak even though they frequently did not succeed in achieving copula. The females were not controlled relative to any variation that may have occurred in their sex drive; however, a female's acceptance or rejection to mating is expressed only after the male is attempting to enter tandem. Thus this variable seems unlikely to influence these observations. Once a male's preference is known, he may be placed with females of the less-preferred type where he shows no desire to mate; however, if males are held in isolation up to four days, the preference breaks down if then offered the less-desirable female and matings are attempted. The choices shown per individual male during the five day tests were completely one-sided except the following. Two F_1 I. damula males, from 211 tested, selected one morph three times and the alternate morph twice. These two males were sibs. Preference was completely fixed among the 125 F_1 I. demorsa males tested. The crosses analysed below include progeny from five I. damula and three I. demorsa matings. Breeding stocks originated from the Bosque Del Apache National Wildlife Refuge 16 miles south of Socorro. Virgin females were mated with six males giving one multiple mating to each female morph in both species. The results are given in Tables 6 and 7. F_1 segregation of male mating-types and female morphs was scored giving two ratios for each cross. No phenotypic ratio differs significantly from either 3:1, 1:1 or 0:1 distributions.

In the earlier breeding tests for female morphs, the homozygous recessive, hh, proved to be phenotypically recognizable while F_1 data were necessary to distinguish between the h^+h^+ and h^+h classes. Thirty-seven crosses were required to confirm the presence of the three genotypes for the h locus in both males and females of the parental stocks. Extending these methods to the eight crosses reported here, parental I. damula males 1, 2 and 4 were apparently heterozygous at the h locus as were the three heteromorphic I. damula female parents. The progeny from male 4 and the andromorphic female parent further identify his genotype as h^+h . Male 3 of I. damula is judged as homozygous recessive. Likewise, males 1 and 2 and both heteromorphic females in the parental I. demorsa are recognized as heterozygotes. The h^+h class is shown for male 2 by the F_1 segregation from the andromorphic female parent. The additional data in Tables 6 and 7 bear on the segregation of male offspring. The F_1 male mating-types

not be as strong as the above tests suggest; however, the likelihood of a male experiencing even a day without female company is low in my judgement. These insects occur in large numbers and furthermore often appear in local aggregations about a habitat. While the sex ratio of mature individuals has not been quantitatively estimated, both sexes are encountered readily and males and females seem likely to "rub shoulders" many times during an average day. Surely, the preferences occur to some extent in the natural colonies. As a consequence, the mating system could lead to a higher degree of homozygosity for a single locus than generated by random mating. From above data and proposed explanation, one may wonder how the dimorphism has maintained itself. Two species, I. posita and I. preparva, in the U. S. are of special interest in this context as only heteromorphic and andromorphic females exist respectively. Perhaps one morph can go to fixation if conditions of allopatry and sympatry are sufficiently stable. Genotypes from randomly collected female larvae and mature males used for breeders revealed earlier that most individuals are heterozygotes at the h locus and perhaps possess some intrinsic advantage. An additional association with fitness is suggested by the fact that the same dimorphism is, at least for some ischnurans, unlikely to fit the explanation reached here. For instance, the genus has a near-cosmopolitan distribution and the dimorphism is found nearly world wide in some species. The cosmopolitan distribution of the dimorphism throughout the genus furthermore implies that the trait has had a long history. The dimorphism is perhaps part of a complex mechanism and may interact with a number of selection agents. If so, a unit of linked genes, each with different functions, may best describe the h "locus". Finally, it is tempting to speculate that only one locus is involved with a sexually dimorphic pleiotropism expressed in both female morphs and male preferences.

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