THE INHERITANCE OF FEMALE DIMORPHISM IN THE DAMSELFLY, ISCHNURA DAMULA

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THE genetics of sex-limited polychromatism in natural populations has been investigated in only a few animals (see review by FORD 1961), none of the known instances including Odonata (damselflies and dragonflies). It is the purpose of the present paper to report the results of a breeding study of female dichromatism in *Ischnura damula*. This appears to be a case of balanced polymorphism, but its adaptive significance is not yet understood.

The dimorphic forms of *I. damula* have been described by WALKER (1953), and the color pattern differences on the dorsal synthorax are shown in Figure 1. One of the female types closely resembles the male. Homoeochromatic, isochromatic, and andromorphic are the terms applied to the male-like form, and the other form has been termed heterochromatic or heteromorphic in the literature of the Odonata (TILLYARD 1917; FRASER 1933; NEEDHAM and HEYWOOD 1929; LONGFIELD 1960). Although homoeochromatic and heterochromatic are the terms most frequently employed by entomologists, andromorphic and hetermorphic have been adopted in this paper to avoid possible confusion with the cytogenetic meaning of the word heterochromatic.

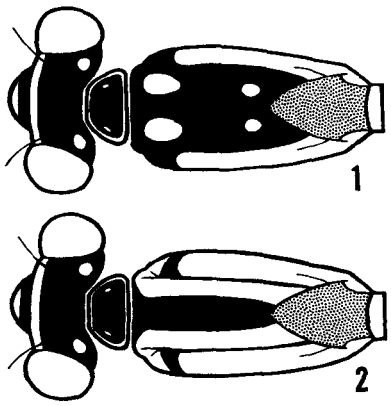

FIGURE 1.—Comparison of dark and light pigment patterns of the dorsal synthorax illustrating female dimorphism in *Ischnura damula*. (1) Andromorphic and (2) heteromorphic females respectively.

MATERIALS AND METHODS

Four New Mexico populations occurring in two distinct ecological surroundings have been studied. The stations were the Federal Fish Hatcheries at Elephant Butte Dam and the Bosque Del Apache National Wildlife Refuge in the Rio Grande valley, and the Tularosa River headwaters and Mangus Springs in the southwestern mountains, five miles north and 16 miles west of Aragon. All breeding stock originated from the Bosque Del Apache Refuge.

Culture procedures and techniques for inducing mating and oviposition under laboratory conditions will be published in detail elsewhere. Constant laboratory conditions were obtained with a Percival Plant Growth Environmental Chamber, Model PGC-78. Illumination was with incandescent light approximating 850 lumen. Breeding data were obtained from known virgin females reared from larvae, bred in the laboratory, and provided with separate oviposition chambers. Larvae from each parental female were reared to emergence while isolated from other stocks, and each female offspring was scored for color pattern phenotype.

RESULTS AND DISCUSSION

Relation of age and environment to expression: Development of adult color and pattern requires a time interval following emergence that varies with species and ambient temperature. Most species of Odonata are pale orange with little or no pattern at emergence, and the adult color pattern is reached through a stepwise sequence of pigment deposition. Once the adult condition exists, further change may occur in two ways. First, migration of intracellular pigment in response to temperature may occur producing a "bright" and "dark" phase (O'FARRELL 1963). This variation is a change in color intensity and does not affect species-specific pigment patterns. Second, processes associated with aging often produce an exudate, pruinesence, that covers parts of the body obscuring color and pattern in the cuticle. Pruinesence is most frequently encountered in odonate males and may function in sexual recognition (JOHNSON 1962a, b). The phenomenon in Ischnura however affects females to a greater degree than males, and changes both female forms of *I. verticalis* and *I. demorsa* to a uniform bluish-white in later adult life; however, distinction of the two forms in *I. damula* is not obscured in this way (WALKER 1953). Except for the temperature effect reported by O'FARRELL, the above variations in color pattern are a function of age. Most Ischnura populations are heterogenous with respect to age, and previous studies of the genus were not designed to reveal the relation of age or environment to the incidence of the two female forms.

Both environment and age were considered in the present study. Environmental effect was evaluated by comparing the population composition existing in natural habitats with adults obtained from larval stocks collected at the study sites and reared under laboratory conditions. Age effect was determined by observing both forms from emergence through a period equal to or greater than the life expectancy in nature, and including the period of mating.

Adults from the study sites were sampled monthly during the greater part of the flight season for the proportion of andromorphic females. These data are given in Table 1. The samples are considered to be random since the size of individuals in this species requires a specimen to be in hand in order to be scored, and no data exist suggesting differential behavior between the forms. The following dis-

TABLE 1

Percent andromorphic females at study sites during three to four generations. Ratio in parenthesis gives andromorphic to total female sample numbers respectively

Date	Study site			
	Bosque Del Apache	Elephant Butte Dam	Tularosa River	Mangus Springs
August 1962	14.3 (9/63)	11.3 (6/53)	17.5 (7/40)	16.6 (8/48)
September 1962	13.5 (7/52)	11.9 (5/42)	15.8 (6/38)	15.7 (8/51)
October 1962	10.3 (6/58)	14.3 (6/42)	15.6 (5/32)	14.3 (6/42)
April 1963	11.1 (8/72)	9.2 (5/54)	No data	No data
May 1963	14.0 (7/50)	11.7 (7/60)	No data	No data
June 1963	13.8 (9/65)	12.1 (7/58)	16.6 (5/30)	15.4 (8/32)
July 1963	12.5 (6/48)	12.0 (6/50)	16.3 (7/43)	15.4 (6/39)
August 1963	13.7 (7/51)	14.3 (6/42)	15.0 (6/40)	15.9 (7/44)
September 1963	11.6 (5/43)	12.8 (5/39)	15.6 (5/32)	16.2 (6/37)

cussion assumes that the populations are at equilibrium relative to frequencies of the two forms, and the generation number is therefore significant for the time shown in Table 1. The generation interval for *I. damula* has been determined from laboratory growth data (to be published elsewhere); these data indicate that three to four generations existed in the field during the sampling period.

Temperature and light duration are the physical parameters having most effect on Odonata (CORBET 1963). Larval samples requiring up to four instars for emergence were collected from each habitat and cultured under the three conditions listed in Table 2, where the percentage of andromorphic females obtained from each of the habitat stocks is given. No significant differences exist between the frequencies in the natural population (Table 1) and the laboratory population (Table 2).

The females emerging from these cultures could be scored for color type within 30 minutes after ecdysis. Many of these females, of both forms, survived in the laboratory up to six days, feeding, breeding, and laying fertile eggs. Distinctness of the dimorphic trait did not change during this period, and the six-day interval is probably longer than the life expectancy in nature. Life expectancy has been

TABLE 2

Percent andromorphic females obtained from stocks collected at study sites and reared under specific laboratory envirnoments. Ratio in parenthesis gives andromorphic to total female sample numbers

Light duration and temperature	Study site			
	Bosque Del Apache	Elephant Butte Dam	Tularosa River	Mangus Springs
24 hr light, 22°C	12.5 (4/32)	11.1 (4/36)	15.8 (6/38)	15.4 (4/26)
1 hr light, 29°C	10.7 (3/28)	11.1 (3/27)	15.6 (5/32)	12.7 (4/31)
Alternating 8 hr periods, 19° to 29°C	12.1 (4/33)	11.4 (4/35)	17.2 (5/29)	16.7 (4/24)

determined to be approximately three days for an ecologically similar species (BICK and BICK 1963).

These observations show that the two female types in *I. damula* are not representative of stages in an individual's maturation, and are in the same proportions both in natural habitats and when cultured during the last four instars in different laboratory environments. The two patterns are therefore most likely innate traits with high heritability.

Sex-controlled expression: Insect characteristics occurring in only one sex and contributing to physiological and morphological sex distinction can apparently be determined by genic balance between autosomes and sex chromosomes (CREW 1954); however, characters having unequal frequencies between the sexes could result from other mechanisms. These possibilities include (1) differential lethality between the morphs in one sex prior to scoring, (2) two distinct stable gene equilibria existing for each sex because of male and female fitness values that differ both in direction and degree (OWEN 1952, 1953), (3) higher frequencies of a recessive sex-linked trait in the heterogametic males than in the females, which must be homozygous for the recessive (LI 1955), and (4) expression of either autosomal or sex-linked traits in only one sex (termed sex-controlled [FORD 1961] or sex-limited inheritance).

On the basis of the proportions of the two forms in female *I. damula*, alternative (1) above requires a male mortality large enough to be detected in the sex ratio (assumed to be close to unity at fertilization). The sex ratio for the progeny of 14 crosses reared from eggs is given in Table 3. With one exception, females are more frequent than males for all crosses, but the female proportions are not large enough to explain the complete absence of heteromorphic males. Alternatives (2) and (3) would require heteromorphic males to exist in frequencies that could be detected by sampling in equilibrium populations. Such males have never been observed or reported. If the data in Table 1 represent populations at equilibrium with respect to the color dimorphism, (2) and (3) are not applicable to *I. damula*. In view of these observations, the occurrence of the second type only in females suggests sex-limited or sex-controlled inheritance.

Single-allelic-pair mechanism: The dimorphism in *I. damula* is probably due to alleles at a single locus, rather than being polygenic in its basis; this is indi-

TABLE 3

Numbers of adult females and males resulting from 14 laboratory crosses. Female percentage given in parenthesis

Cross number	Females	Males	Cross number	Females	Males
1	39 (56.6)	30	8	43 (57.3)	32
2	41 (56.9)	31	9	40 (56.3)	31
3	38 (55.8)	30	10	45 (55.6)	36
4	28 (43.9)	36	11	44 (55.0)	36
5	44 (54.9)	36	12	44 (53.7)	38
6	50 (55.7)	40	13	46 (55.3)	37
7	42 (57.6)	31	14	48 (53.8)	41

cated by the occurrence of only two distinct classes, and by the fact that both forms segregate in the progeny of single females in the ratios given in Tables 4 and 5. (Whether the polymorphism involves a single gene difference or a difference in chromosome structure cannot, however, be distinguished from the present data.) The simplest hypothesis is that a single pair of alleles determines the two color phases. A hypothetical model of complementary gene pairs could perhaps be constructed which would fit the breeding data. Such a model would however, require more assumptions than the hypothesis of a single allelic pair, which leads to expectations agreeing closely with the data. In addition, the evolution of dimorphism through complementary gene pairs is considered improbable on theoretical grounds (FORD 1961; SHEPPARD 1961).

Crosses contributing to the breeding data involved a virgin female and a single male per cross; however, one male was used in three crosses, and two males were each used in two crosses, as shown in Tables 4 and 5. Results of the crosses can be summarized as follows: (1) individual females of both phenotypes produced progeny including both morphs; (2) the progeny of andromorphic females could

TABLE 4

Morph ratios in female offspring segregating from heteromorphic female parents. See text for origin of expected values

Fertilized by male No.	Sample size	Female offspring: Percent heteromorphic Observed	(Expected)	Percent andromorphic Observed	(Expected)
1	41	73.2	(75)	26.8	(25)
1	38	100.0	(100)	0.0	(0)
3	44	65.9	(75)	34.1	(25)
4	50	80.0	(75)	20.0	(25)
5	42	78.6	(75)	21.4	(25)
6	43	100.0	(100)	0.0	(0)
7	45	80.0	(75)	20.0	(25)
8	44	100.0	(100)	0.0	(0)
10	48	43.8	(50)	56.2	(50)

TABLE 5

Morph ratios in female offspring segregating from andromorphic female parents. Otherwise similar to Table 4

Fertilized by male No.	Sample size	Female offspring: Percent heteromorphic Observed	(Expected)	Percent andromorphic Observed	(Expected)
1	39	56.4	(50)	43.6	(50)
2	28	39.3	(50)	60.7	(50)
7	40	40.0	(50)	60.0	(50)
8	44	100.0	(100)	0.0	(0)
9	46	0.0	(0)	100.0	(100)

be either 100 percent andromorphic or 100 percent heteromorphic; whereas (3) the progeny of heteromorphic females consisted of only the heteromorphic type when all offspring were alike; (4) when both types occurred in progeny of the same female, the ratios were 1:1 from andromorphic, and 1:1 or 3:1 from heteromorphic females. These data suggest that the andromorphic female is homozygous recessive (hh) and that heteromorphic females are either heterozygous (h^+h) or homozygous dominant (h^+h^+). Since male genotypes were not known when crosses were made, progeny of andromorphic females may be expected to occur in three ratios, 1 dominant:1 recessive, 1 dominant:0 recessive, or 0 dominant:1 recessive. Progeny of heteromorphic females may be expected in the ratios 3:1, 1:1, and 1:0 for dominant to recessive. All of these phenotype ratios were observed, as shown in Tables 4 and 5. A chi-square test, using expected values appropriate to the parental female morph and agreeing best with observed values, showed no significant departure from expectation at the 95 percent confidence level. Expected and observed values are actually quite close for the sample sizes involved. Each of three males, (Nos. 1, 7, and 8) fertilized females of both types. The ratios from these crosses are all compatible with genotypes h^+h, h^+h, and h^+h^+ for Males 1, 7, and 8, respectively.

Segregation of both forms in the progeny of each female morph, as observed above, would not normally occur with a single sex-linked allelic pair. The alleles involved are therefore autosomal.

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SUMMARY

Female dimorphism involving a color pattern in the damselfly *Ischnura damula* does not vary with age and environmental factors. Frequencies existing in natural populations occurred also in population samples cultured under a variety of constant laboratory environments, and the characteristic dimorphic features are retained throughout time intervals approximating life expectancies in nature.

The dimorphism is sex-controlled (sex-limited) in expression. Breeding data indicate that the phenomenon is governed by a single allelic autosomal gene pair. Females with the male-like pattern are homozygous recessive, and females of the other pattern type are heterozygous and homozygous dominant.

LITERATURE CITED

BICK, G. H., and J. C. BICK, 1963 Behavior and population structure of the damselfly, *Enallagma civile* (Hagen) (Odonata: Coenagriidae). Southwestern Naturalist **8:** 57–84.

CORBET, P. S., 1963 *A Biology of Dragonflies.* Quadrangle Books, Chicago.

CREW, F. A. E., 1954 *Sex-Determination.* Wiley, New York.

FORD, E. B., 1961 The theory of genetic polymorphism. pp. 11–19. *Insect Polymorphism.* Edited by J. S. KENNEDY. Roy. Entomol. Soc., London.

FRASER, F. C., 1933 *The Fauna of British India. Odonata.* Vol. 1. Taylor and Francis, London.

JOHNSON, C., 1962a Reproductive isolation in damselflies and dragonflies (Order Odonata). Texas J. Sci. **14**: 297–304. —— 1962b A study of territoriality and breeding behavior in *Pachydiplax longipennis* Burmeister (Odonata: Libellulidae). Southwestern Naturalist **7**: 191–197.

LI, C. C., 1955 *Population Genetics.* University of Chicago Press, Chicago, Illinois.

LONGFIELD, C., 1960 The British species. pp. 11–24. *Dragonflies.* P. S. CORBET, C. LONGFIELD, and N. W. MOORE. Collins, London.

NEEDHAM, J. G., and H. B. HEYWOOD, 1929 *A Handbook of the Dragonflies of North America.* Charles C. Thomas, Springfield, Illinois.

O'FARRELL, A. F., 1963 Temperature-controlled physiological color change in some Australian Damsel-Flies (Odonata: Zygoptera) Australian J. Sci. **25**: 437–438.

OWEN, A. R. G., 1952 A genetical system admitting of two stable equilibria. Nature **170**: 1127–1128. —— 1953 A genetical system admitting of two distinct stable equilibria under natural selection. Heredity **7**: 97–102.

SHEPPARD, P. M., 1961 Recent genetical work on polymorphic mimetic Papilios. pp. 20–29. *Insect Polymorphism.* Edited by J. S. KENNEDY. Roy. Entomol. Soc., London.

TILLYARD, R. J., 1917 *The Biology of Dragonflies (Odonata or Paraneuroptera).* Cambridge University Press, Cambridge.

WALKER, E. M., 1953 *The Odonata of Canada and Alaska.* Vol. 1. Toronto University Press, Toronto.