SHORT PAPERS

INTERACTIONS OF DIFFERENTIATED PRIMARY SEX FACTORS IN *CHIRONOMUS TENTANS*[1]

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ABSTRACT

Different populations of *Chironomus tentans*, possibly representing geographically isolated races, have two differentiated genic mechanisms of sex determination involving either a dominant male-determining factor in the left arm of chromosome 1 or a dominant female-determining factor at the right tip of chromosome 1. In crosses between these populations, the male-determining factor is epistatic to the female-determining factor. No evidence of intersexuality has been found in such crosses.

SEX determination by primary sex factors, while commonly postulated as a state preceding the differentiation of sex chromosomes (KERR 1962; MITTWOCH 1967), is rarely encountered among existing animal species. The widely cited case of *Bracon juglandis* (WHITING 1939) provides evidence for the control of sex by pairs of elements which are often designated "sex alleles," but there is reason to think that these elements are homologous segments with numbers of loci complementing or interacting (WHITING 1943). The demonstration of genic sex determination in Chironomus was first made by BEERMANN (1955) in his report of male-limited inversion sequences in several populations of *Chironomus tentans* and *Chironomus pallidivitattus*. In spite of his having designated these sequences "Y chromosomes," BEERMANN was often able to show the separation of a genic male-determiner from the inversion by crossing over.

North American populations of *Ch. tentans* give evidence of differentiated genic control, where one race appears to carry a dominant male-determining factor and another geographically distinct race carries a dominant female-determining factor (THOMPSON 1971). The evidence for differentiated modes of sex determination (THOMPSON 1971, and new data) is summarized in Table 1. Iowa populations from several localities invariably had a male-determiner, *Md*, which was inseparable from inversion sequence 1L (designation of ACTON 1959) in the left arm of chromosome 1. The initial reason for assuming a single primary factor was simply that BEERMANN had described several such factors at various positions, usually at the tips of arms, in the several European populations studied.

Populations from Wisconsin and Ontario, on the other hand, had a demonstably genic female-determiner, *Fd*, at the tip of the right arm of chromosome

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TABLE 1

Primary sex factors in natural populations

Locality	Heterogametic sex	Associated inversion sequence	Map position
Dickinson Co., Iowa	male	1L	inseparable from inversion
Hamilton Co., Iowa	male	1L	inseparable from inversion
Cerro Gordo Co., Iowa	male	1L	inseparable from inversion
Madison, Wisconsin	female	1RaRe	right tip–7% C.O. with inversion
Ottawa, Ontario	female	1RaRe	right tip–11% C.O. with inversion

1. While this factor was initially associated with inversion sequence 1RaRe, its exact position was eventually determined by the recovery of occasional crossovers separating it from that sequence.

In order to determine whether these patterns typify two sexually isolated races, as well as to examine an unusual mechanism of sexual differentiation, all combinations of the primary factors and their alleles were made. The primary factors appear to act (or interact) without any substantial interplay with other elements of the genome. The *Md* factor is epistatic to the *Fd* factor.

MATERIALS AND METHODS

The interaction of male and female-determining factors was tested by crosses of Dickinson males (dominant male factor *Md* inseparably linked to sequence 1L and paired with sequence 1Lk; homozygous for sequence 1Ra in the right arm of chromosome 1) with Madison females (dominant female factor *Fd* linked to sequence 1RaRe and paired with sequence 1Ra: homozygous for sequence 1Lk in the left arm.) For a correlation of sex with chromosomal constitution, last-instar larvae were sexed by an examination of their developing gonads as described by WUELKER and GOETZ (1968). Salivary glands were squashed in lacto-acetic orcein following dissection in 0.7% saline solution and fixation with acetic-alcohol. Chromosome sequences were typed according to the description and designations of ACTON (1959).

The fertility of F_1 adults was tested by matings to the Madison stock. Developing embryos can easily be observed and counted (because of the regular geometrical arrangement of eggs in the clear gelatinous egg mass) up to the first larval instar. The criteria for fertility were a normal number of eggs (400 plus) in the egg mass, and emergence of at least 50% as larvae.

RESULTS AND DISCUSSION

A correlation of the four "sex chromosome" combinations with sex and fertility of progeny is shown in Table 2. The results show strikingly that each specific combination of left- and right-arm sequences of chromosome 1 gave a single, clearcut response in sexual development. A balance mechanism, involving shared control of sexual differentiation by the putative primary loci and by background factors, might be expected to produce some intersexuality or a continuum of sexual types in racial admixture as in the case of *Porthetria* (=*Lymantria*) *dispar* (GOLDSCHMIDT 1930). Here, however, as in BEERMANN's (1955) crosses of

TABLE 2

Sex factor combinations from inter-racial crosses

Chromosomal constitution	Genotype if non-recombinant	Larvae analyzed	Sex	Fertility of adult progeny
1L 1Ra/1LK 1RaRe	Md/Fd	51	all male	31/34 males were fertile in
1L 1Ra/1LK 1Ra	Md/—	31	all male	matings to normal females
1LK 1Ra/1LK 1RaRe	—/Fd	39	all female	16/21 females deposited
1LK 1Ra/1LK 1Ra	—/—	45	all female	fertile egg masses

Ch. tentans × *Ch. pallidivitattus*, the primary factors seem to maintain their primacy in mixed genetic backgrounds. While positive identification of individual genotypes could not be made in the fertility tests, the number of infertile adults in the hybrid group was as low as it normally would be in a standard series of matings. This finding, together with the observation of normal gonadal development in larvae of all four genotypes, makes it very unlikely that sterile or intersexual progeny occur in substantial frequencies.

It is especially interesting that in the combination of strong male and female factors (*Md/Fd*), sexuality is male and fertility is essentially normal. Epistatic female determination has been associated with a differentiated sex chromosome in *Bombyx mori* (Tazima 1943), and epistatic male determination with the differentiated Y chromosome of the calliphorid *Phormia regina* (Ullerich 1963). In such cases, combination of the Y with two or more X chromosomes gives normal Y sexuality. The present case is somewhat unique, however, in showing an epistatic interaction between major genic factors of opposite tendency and at different loci. The equally clearcut femaleness of our category lacking prime factors (—/—) indicates a strong potential in sex determination by other elements of the genome, but these appear to play no determinative part when *Fd* or *Md* are present.

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