## References and Notes

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## Unique Incompatibility System in a Hybrid Species

*Abstract.* All males of the weevil *Pissodes terminalis* Hopp. are heterozygous for an autosomal fusion ($cc \rightarrow C$); all females are homozygous ($CC$). A mechanism is operative whereby union of like gametes gives rise to females and of unlike gametes gives rise to males, and union of half-alike gametes is totally excluded. The perpetuation of this sexual dimorphism of karyotypes following crossing implicates *P. yosemite* Hopk. in the hybrid origin of *P. terminalis*.

Chromosomal polymorphism is said to be transient if it leads to changes in the genetic composition of a population. It is balanced if the structural heterozygotes exhibit heterosis. Heterosis usually expresses itself as a result of differential mortality, with more heterozygous adults comprising the population than are expected on the basis of the binomial square rule, or by greater fecundity, longevity, or sexual activity of the heterozygotes. The most extreme case of balanced polymorphism we can envisage would be achieved by the incorporation of a balanced lethal system that would eliminate both homozygous types. With a consequent 50 percent wastage in each generation, it is understandable that balanced lethal systems have not been encountered in natural populations of animals.

In *Drosophila tropicalis* Burla & da Cunha, Dobzhansky and Pavlovsky (*1*) have demonstrated that populations occur in Honduras in which the fitness of the homozygotes approaches zero, with about 90 percent of the adults being heterozygotes and most of the mortality occurring in the egg stage. Despite this, as judged by the relative abundance of *D. tropicalis* and three other siblings in the *D. willistoni* group, the populations were found to be "remarkably prosperous."

A radically different situation, which appears not to be attributable to differential mortality, prevails in the weevil *Pissodes terminalis* Hopping (*2*). In this case all 218 males examined cytologically, from populations in California, northern British Columbia, the Yukon Territory, and Saskatchewan, proved to be heterozygous and all 41 females, from the same localities, proved to be homozygous for the '$C$' metacentric chromosome (the fusion equivalent of two acrocentrics, '$c^1c^2$') (*3*).

In other words, the $C$-chromosome complex behaves in heredity as though it were concerned with sex determination, the two $C$ chromosomes determining femaleness, the $Cc^1c^2$ chromosomes, maleness. It is, however, perfectly obvious that it does not have this function. The highly distinctive *Pissodes* $X$ and $Y$ sex chromosomes, plainly evident in primary spermatocytes, segregate at random relative to the disjoining $C$ and $c^1c^2$ chromosomes (*2*). Males may, therefore, be symbolized $XYCc^1c^2$ and females $XXCC$.

Preliminary egg sterility tests aimed at discriminating between selective fertilization (which would yield no inviable eggs) and a lethal system (which would eliminate half the theoretical recombinants) imply that multiple insemination occurs, and that it is through fertilization being selective that all males are heterozygous and all females homozygous for the $C$ metacentric (*4*). The striking difference from the classical *Oenothera lamarkiana* device, in which both homozygous categories fail to materialize, resides, of course, in the unique autosomal dichotomy into heterozygous males and homozygous "metacentric" females (that is, to the total exclusion of $CC$ males and $Cc^1c^2$ females). Unless the role of differential mortality is deferred until later in development, this attests the existence in *Pissodes terminalis* of a cytogenetic system entirely new to genetics: one that permits the union of totally dissimilar gametes ($XC + Yc^1c^2$) to give rise to males and of similar ones ($XC + XC$) to give rise to females, but completely debars the union of gametes that are only half alike ($XC + YC$ and $XC + Xc^1c^2$).

The diploid chromosome numbers observed in *P. terminalis* have been consistently 28 in the female and almost always 29 in the male. But the presence in a California population of a male heterozygous for an '$A$' metacentric and of another homozygous for the $a^1a^2$ acrocentrics and heterozygous for a '$B$' metacentric, both in addition to the invariable $Cc^1c^2$ complex, reveals a potential extending the diploid range to 34 (*2*). The lower limit, 28, is constant for both sexes of the California species *P. yosemite* Hopkins; the upper potential limit, 34, is restricted, so far as is known, to the eastern chromosomally monomorphic *P. strobi* Peck and to its western siblings or geographic races, *P. engelmanni* Hopkins and *P. sitchensis* Hopkins. Thus it is not immediately apparent whether the floating variants in *P. terminalis* are to be attributed to chromosomal polymorphism—the presence of structural rearrangements that originated within *P. terminalis* by chromosome mutation—or to introgressive hybridization between 28 and 34 chromosome-numbered species.

Two methods of approach to the problem suggested themselves. First, it was reasoned that if the system of permanently heterozygous males and permanently homozygous females could be synthesized by crossing a species with 28 chromosomes, namely *P. yosemite*, by one with 34 chromosomes, namely *P. strobi*, the "Gordian knot" would be effectively cut. Second, it seemed to us evident that if the unique sexual dichotomy of karyotypes could be perpetuated by crossing *P. terminalis*, as the male, to *P. yosemite*, as the female, this would constitute compelling evidence, if not rigorous proof, of the hybrid nature of *P. terminalis*, and, furthermore, would implicate *P. yosemite* in its origin. Both crosses were successful (*5*), but since the former requires the breeding of further generations, the $F_1$ being of necessity heterozygous for all three metacentrics, the following discussion is restricted to the latter cross.

Adults of *P. yosemite* were reared out of ponderosa pine root collars received from California (*6*) and *P. terminalis* adults out of jack pine terminal shoots shipped from Saskatchewan. The cytological constitution of males is readily and accurately determinable at first metaphase of meiosis.

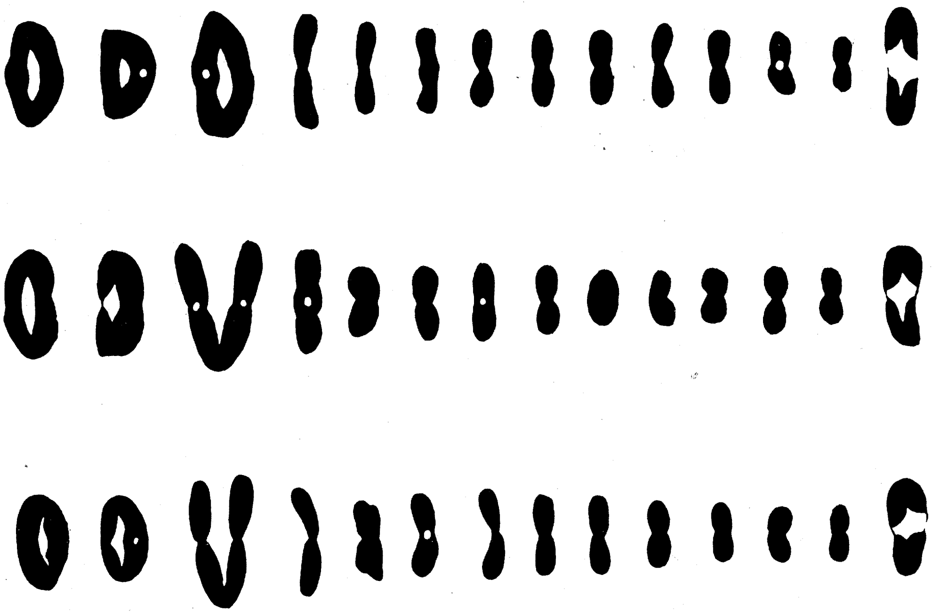

Fig. 1. First meiotic metaphases in males of *P. yosemite* (top), *P. terminalis* (middle), and *P. yosemite* ♀ × *P. terminalis* ♂ (bottom). The three complements are tracings from *camera lucida* drawings, the configurations having been aligned and rearranged, from left to right, in the sequence: *A, B, C*, 10 rods, and *XY* (about × 3000).

Female counts are made most easily from oogonial mitoses, particularly after feeding starved adults on the drug colchicine (0.1-percent aqueous solution in honey) in order to arrest cell division at metaphase. They are less reliable than those for males; in general, the number of large metacentrics was taken as diagnostic or confirmatory.

Maternal *P. yosemite*: the chromosome constitution of females was indirectly established by examination of the spermatocytes of three males. At first metaphase they showed three large ring-shaped bivalents (*A, B*, and *C*), 10 rod-like bivalents, and an *XY* sex-determining bivalent (Fig. 1, top), the established complement for *P. yosemite* (*2*). As in all species of *Pissodes* so far examined, the number of chiasmata never exceeds one per major chromosome arm, that is, two in rings and one in rods.

Paternal *P. terminalis*: All 21 males examined from Saskatchewan were identical in having *A* and *B* rings, a *C* trivalent, 10 rods, and the *XY* pair (Fig. 1, middle). All 18 females had 28 chromosomes, or at least the six expected metacentrics. The sample is, therefore, sexually dimorphic as to karyotype and hence typical of *P. terminalis* (*2, 7*).

$F_1$ hybrids: Ten of the females and all 26 males were examined cytologically. Oogonial metaphases showed 28 chromosomes, the six diagnostic large metacentrics, or both. All males had 29 chromosomes, which at first metaphase formed *A* and *B* rings, a *C* trivalent, 10 rods, and an *XY* bivalent (Fig. 1, bottom). As in both parental species, failure of chiasma formation between one pair of arms in the potential rings was common (about 32 percent). Failure of pairing resulting in two univalents was rare (about 1 percent), and largely restricted to potential rod bivalents. Trivalent failure, resulting in a bivalent plus a univalent, amounted in frequency to roughly 2 percent. Disjunctional orientation of the *C* trivalent by full metaphase was close to being perfect. Chromatid bridges plus fragments, symptomatic of crossing over in relatively inverted segments, were not observed.

*Pissodes yosemite* females, being chromosomally monomorphic, produce only *XC* eggs. Males of *P. terminalis*, being heterozygous for both the sex chromosomes and the *C* complex (which segregate at random), produce four types of sperm: *XC*, *YC*, $Xc^1c^2$, and $Yc^1c^2$. Of the four possible $F_1$ recombinants, *XXCC*, *XYCC*, $XXCc^1c^2$, and $XYCc^1c^2$, the middle two were not realized as adults.

The substitution of *P. yosemite* females for *P. terminalis* females, therefore, induced no breakdown in the mechanism that assures sexually dimorphic karyotypes. Here again, unless perhaps differential mortality is operative, the union of similar or entirely dissimilar gametes is permitted; the union of those that are only half alike is excluded. This incompatibility system clearly depends on the coadjusted properties of two components, the nucleus of the sperm and the cytoplasm of the egg, properties that are equally expressed by *P. terminalis* and *P. yosemite*. It is therefore concluded that *P. terminalis* is a hybrid "species" and that *P. yosemite* participated in its origin, as is evidenced additionally by the absence of disastrous meiotic effects in our $F_1$ hybrids. This conclusion is further supported by the observations of Drouin *et al.* (*7*): ". . . an origin through hybridization followed by introgression, and subsequent adaptation to different geographical areas, account both for the behavioural properties shared by *terminalis* and *strobi*" [the other putative parent species] "and for the phenotypic differences contrasting geographically remote populations of *terminalis*."

A further cross that should serve to test the validity of this reasoning based on the sexual dichotomy perpetuated by means of *P. yosemite* (female) with *P. terminalis* (male) is the reciprocal (now in culture). Since *P. yosemite* male produces only *XC* and *YC* sperms, the former of which, we may surmise, are alone compatible with *P. terminalis XC* eggs, we expect only unisexual, female progeny to materialize. If so, this will constitute an entirely new type of "sex-ratio" condition (*8*).

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