Recombination frequency and DNA content of the distal part of the second chromosome of *Drosophila hydei* Sturtevant

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Summary. Four visible markers, including a newly isolated one, have been cytologically mapped on the second chromosome of *Drosophila hydei*. Both the frequency of recombination and the amount of DNA between these markers have been determined. From these data the coefficient of exchange has been calculated.

Relatively few autosomal mutations of *Drosophila hydei* are known. Three such markers, namely peach, scarlet and ebony, are located in the distal part of the 2nd chromosome[1]. From a screening of ethylmethane sulfonate treated chromosomes we have isolated a 4th such marker: this allele is recessive and results in eyes that are ovoid in shape (fig. 1) and are dull scarlet in color. We have named this mutation 'oval eye'. We could not find a previous report of a homologous mutation in *Drosophila melanogaster*.

We have located peach, scarlet, ebony and oval eye on the cytological map of the tip of the 2nd chromosome by mapping X-ray induced chromosomal deletions that cover these mutations. The established order of the loci agrees with the results of experiments in which the frequency of recombination between these markers was determined. The loci of these markers and their recombination distances are given in figure 2.

To relate the genetic map units to a physical distance, we have determined cytophotometrically the amount of DNA that separates these loci: squashed polytene chromosomes are Feulgen stained and the absorbtion of the selected region is measured by scanning densitometry. As the haploid DNA content of the 2nd chromosome of *D. hydei* is about 45,000 kilobases (kb), the haploid DNA content of any region of the 2nd chromosome can be calculated by comparing its integrated absorbance with the integrated absorbance of the whole chromosome (for full details, see Derksen et al.[2] and Grond et al.[3]). The amount of DNA that separates the 4 visible distal markers on the 2nd chromosome is shown in figure 2. Hence, 5640 kb of DNA corresponds to 25.0 morgan units, the genetic distance between ebony and oval eye, and the coefficient of exchange (amount of DNA per unit of recombination, see Lindsley and Sandler[4]) is about 2250 bp/0.01 morgan units.

It has been suggested that the coefficient of exchange in *D. hydei* should be lower than that in *D. melanogaster*, since the genetic map of the X chromosome of *D. hydei* is almost twice as long as that of the X chromosome of *D. melanogaster*, yet the 2 X chromosomes should carry about the same amount of genetic information[5,6]. Frei[6] calculates a coefficient of exchange of 3500 bp/0.01 morgan units for the *D. hydei* X chromosome. A similar calculation (total DNA content devided by total map length) for the X chromosome of *D. melanogaster* would yield a coefficient of exchange of about 4500 bp/0.01 morgan units. This value is somewhat higher that the 3600–3800 bp/0.01 morgan units given by Lefevre[7] for the white-forked region of the X chromosome since it would include the centromere and telomere effects. We find a significantly lower coefficient of exchange (i.e. 2250 bp/0.01 morgan units) for the distal part of the 2nd chromosome of *D. hydei*. This coefficient of exchange was constant (see fig. 2) over the region considered (about 13% of the 2nd chromosome) and is thus not likely to be influenced by a telomere effect or the possible presence of a region with exceptional recombination properties. Furthermore, these is little difference between the coefficient of exchange of the X chromosome and the autosomes in *D. melanogaster*[4] and it is unlikely that such a difference would exist in *D. hydei*. The discrepancy between our data and the coefficient of exchange calculated for the *D. hydei* X chromosome by Frei[6] is probably due firstly to the uncertainty in his estimate of the DNA content of the euchromatic arm of the X chromosome and, secondly and more importantly, to the fact that the recombination frequency is not constant along the length of the chromosome but is rather lower near the centromere. This means that the average coefficient of exchange measured over a whole euchromatic arm will be higher than the coefficient of exchange measured over a distal segment, as we did.

Our data can be directly compared with the data of Lefevre[7] since both deal with a chromosomal segment in which the recombination frequency is not influenced by either telomere or centromere effects. We conclude then that the frequency of recombination per unit of DNA in *D. hydei* is 1.6 times as high as that found in *D. melanogaster*.

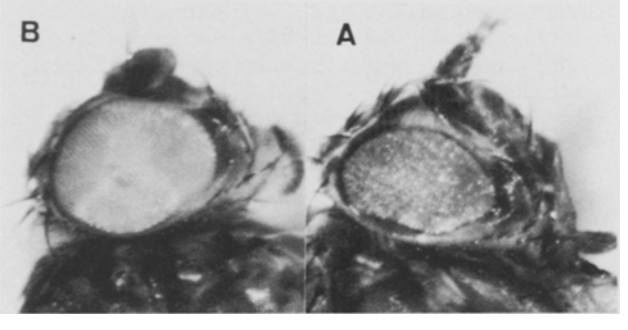

Figure 1. The oval eye mutation (oo) of *D. hydei*. *A* Eye of oval eye phenotype fly. *B* Eye of wild type fly.

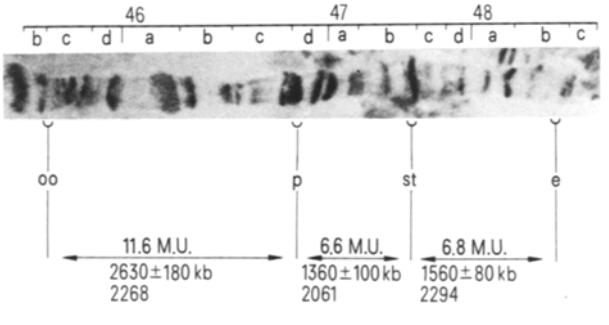

Figure 2. Markers and their distances in the distal end of the 2nd chromosome of *D. hydei*. The chromosome regions are numbered following Berendes' map[8]. Values for the coefficient of exchange (c.e.), for the frequency of recombination in morgan units (MU) and for lengths of DNA in kilobases (kb) ± SD are given for the adjacent loci.

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Evidence for an alarm substance in *Polistes canadensis*[1]

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Summary. In this tropical social wasp the odor of venom elicits an alarm response, reduces the threshold for attack, and acts as an attractant. Attack is released visually; dark colored objects were shown to be more effective releasers than were moving objects.

In recent years chemical communication of alarm has been documented in a wide range of higher social Hymenoptera[2,3]. In the primitive social wasp genus *Polistes*, however, evidence for alarm pheromones has not been found. Maschwitz tested body substances and found none that would elicit alarm in *Polistes dubius* Kohl[2]. In addition, Freisling showed for *P. gallicus* (L.) and *P. nimpha* (Christ) that wing buzzing by a disturbed wasp would excite others on the nest to an alert posture, whether the buzzing wasp was intact or had had its abdomen removed[4]. It has therefore been concluded that these wasps rely on communication of alarm via substrate vibration[4,5]. In this paper I report the discovery of chemical communication of alarm in *Polistes canadensis* (L.). This is the first evidence of a pheromone in this primitively eusocial wasp genus.

Polistes canadensis occurs from Arizona through Central and South America to Paraguay, Bolivia, and northern Argentina[6]. At Santarém, Pará, Brazil, where the present study was carried out, colonies are locally extremely common and have the unusual habit of building multiple combs, rather than just one as is typical of the genus[7]. The observations and experiments reported below were performed on colonies in situ.

Wasps on the nest respond to movements of nearby large objects with mild alarm behavior: they turn to face the disturbance, raise the anterior end of the body, wave the forelegs, spread and elevate the wings, and increase ventilatory pumping of the gaster. If the disturbance is more violent (e.g. if wasps are pulled from the combs, or if combs are removed from the nest), alarm intensifies: the wings are buzzed, the sting chamber is held slightly open, and the tip of the gaster is flexed to one side. This may be followed by attack: one or more wasps fly at the intruding object and attempt to sting it.

I found that once an object has been attacked, merely passing it upwind of the nest elicits a wave of renewed attacks. If a female is held in a pair of forceps, she struggles and attempts to sting. When such a struggling wasp is held upwind of a nest, the colony becomes strongly alarmed. These observations suggest the presence of an alarm pheromone. The following experiment was carried out to test this possibility and to determine the effectiveness of color and movement in eliciting attack behavior.

The search for the alarm pheromone was made by crushing female body parts onto a piece of white filter paper folded around the end of a 75 cm dowel and held in place by a paper clip. The paper was then presented upwind of an unalarmed colony. Tested materials included:

1. Venom sac. 1 per trial.
2. Dufour gland. 2 per trial.
3. Hemolymph squeezed from gaster after removal of sting apparatus and venom sac. 2 wasps per trial.
4. Glacial acetic acid. Several drops.
5. Formic acid. Several drops.
6. Clean, dry filter paper (control).

2 wasp equivalents of Dufour gland and hemolymph were used per trial because each yielded smaller quantities of material than did the venom sac. The purpose was simply to determine whether each body part contained alarm pheromone or not, and not to determine the relative response per wasp equivalent.

Responses by *Polistes canadensis* to 4 visual models and the filter paper bearing the tested substance. Data are total numbers of wasps responding to each object. Numbers of trials yielding a response are given in parentheses. All responses to the visual models were stinging attacks. Most responses to the odor source were calm inspection

Tested substance	No. trials	Visual models				Filter paper
		Moving		Non-moving		
		Dark	Light	Dark	Light	
Clean control	9	0	0	0	0	0
Acetic acid	5	0	0	0	0	0
Formic acid	5	0	0	0	0	0
Hemolymph from gaster	6	0	0	0	0	0
Dufour gland	5	2(1)	0	0	0	0
Venom sac	5	64(5)	0	2(1)	0	5(3)
Venom sac and sting apparatus from:						
2 wasps	11	79(6)	3(1)	12(4)	0	27(8)
3 wasps	5	50(5)	2(1)	10(4)	0	16(5)