

dependence *white* expression as was described by Hazelrigg and Petersen (1992). So, we concluded that the same regulatory flanking elements could be responsible for *white* repression in our case. The only new insertion (A) had the classical "salt and pepper" phenotype (Figure 2), modified by lower temperature and removing Y chromosome such as a classical heterochromatin-induced PEV. Its cytological position is 24D1-2. To be sure that "salt and pepper" phenotype in 24D1-2 region is not a result of *white* gene damage or mutation we have generated new transpositions of the A^R4-24 element from 24D1-2, using this mosaic line, by analogy with outline described before. Relocating the gene should result in a wild-type eye color at most new positions if its mutant phenotype is due to a position effect, but not if it is due to a mutation intrinsic to the gene. 44280 males were screened, 2323 wild-type revertants, 2 stable repression of *white* expression were registered and 54 "salt and pepper" lines with different extent of mosaicism were isolated (Table 1). Thus, position effect in the 24D1-2 regions is very probably caused by adjacent to A^R4-24 element genomic DNA, since transposon is placed in a distance of 15 cytological division from nearest heterochromatin and there are no visible reasons for *cis*- or *trans*- interactions (Figure 3).

Acknowledgments: The work was supported by grants from "Frontier genetic program of Russian Federation" and RFBR (99-04-49270, 96-15-97749). I thank Dr. E.S. Belyaeva and Prof. I.F. Zhimulev for comments on the manuscript.

References: Hazelrigg, T., and S. Petersen 1992, *Genetics* 130: 125-138; Levis, R., T. Hazelrigg, and G.M. Rubin 1985, *Science* 229: 558- 561; Robertson, H.M., C.R. Preston, R.W. Phillis, D.M. Johnson- Schitz, W.K. Benz, and W.R. Engels 1988, *Genetics* 118: 461-470; I.F. Zhimulev 1997, *Adv. Genet.* 37: 1-555.

Proportions of *Drosophila melanogaster* and *D. simulans* in eastern Australian populations.

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Conventional wisdom among drosophilists states that typical relative abundances of *Drosophila melanogaster* and *D. simulans* vary with latitude and with indoor vs outdoor feeding/breeding site, as well as with season and temperature. In March and April of 1997, I collected *Drosophila* flies at 40 localities near the coast of eastern Australia, in New South Wales, Victoria and Tasmania. Data were recorded on the numbers of both sexes of *D. melanogaster* and *D. simulans* captured at each site, and on characteristics of the site itself. The data and some analyses are presented here to contribute to the literature on the ecology of these species.

The collections were intended to sample *D. melanogaster* populations in order to determine the state of the previously described clinal pattern in P-M hybrid dysgenesis (Boussy, 1987; Boussy and Kidwell, 1987; Boussy *et al.*, 1988). Latitudes of collection sites were determined using a hand-held global positioning

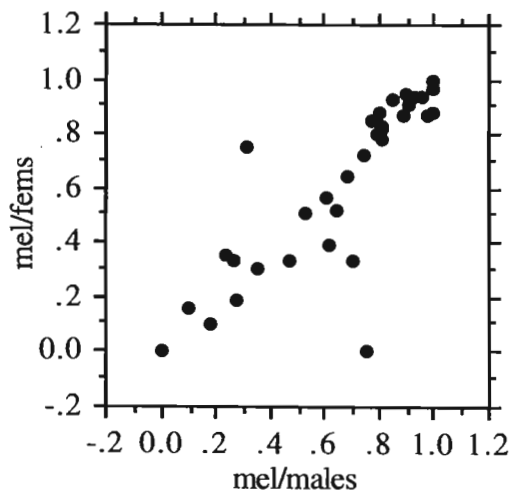


Figure 1. Proportion of *D. melanogaster* in samples of males vs. samples of females.

satellite receiver (Garmin GPS XL 45). Flies were collected by sweeping at sites attractive to *Drosophila* flies (e.g., discarded fruit in orchards or vineyards, waste bins in fruit-processing sheds, displays and discard bins in fruit and vegetable shops, pomace heaps at wineries, a home compost heap), or from buckets containing chopped bananas and live yeast as bait. I used an insect net with a tubular trap in the bottom ("Drosophila Net," Wards, cat. No. 10W0495; modified by stitching the trap smaller to snugly fit a 25 mm vial). The net was fitted to a standard insect net frame ("Collapsible Net," BioQuip, cat. No. 7115CP), used with a 24 inch extension handle. Flies were swept over sites or bait buckets, and collected from the net into 25 mm vials containing previously prepared standard yeast-agar-treacle-maize meal *Drosophila* food. The vials were kept cool until sorting (within a few hours) in a ca. 0.1 m³ styrofoam container with ice or cold water in containers. Flies were anesthetized with ether and

Table 1. *D. melanogaster* and *D. simulans* in eastern Australian collections.

Collection locality	in/out	°Slat	mel males	sim males	mel females	sim females	mel/ males	mel/ females	mel/ both
Coffs Harbour, NSW	o	30.244	54	48	29	28	.529	.509	.522
Nambucca Heads, NSW	i	30.641	43	10	18	4	.811	.818	.813
Kempsey Farmers' Market, NSW	i	31.081	15	4	16	4	.789	.800	.795
Hasting River Winery, NSW	o	31.300	57	102	40	91	.358	.305	.334
Wauchope, NSW	o	31.536	23	205	33	179	.101	.156	.127
Laurieton, NSW	i	31.648	60	14	45	9	.811	.833	.820
Taree, NSW	i/o	31.913	9	5	12	11	.643	.522	.568
Forster, NSW	i	32.179	54	16	41	7	.771	.854	.805
Woolton, NSW	o	32.263	•	•	400	44	•	.901	.901
Bennett's Green, NSW	i	32.998	56	13	40	11	.812	.784	.800
Kanwal, NSW	i	33.264	28	32	11	22	.467	.333	.419
Berkeley Vale, NSW	o	33.325	•	•	73	3	•	.961	.961
Woy Woy, NSW	i	33.488	31	20	16	12	.608	.571	.595
Glebe, NSW	o	33.875	•	•	3	67	•	.043	.043
Berry, NSW	i	34.775	36	4	36	2	.900	.947	.923
Bomaderry, NSW	o	34.846	9	42	4	35	.176	.103	.144
Ulladulla, NSW	i	35.364	57	7	13	2	.891	.867	.886
Batehaven, NSW	i	35.733	23	0	8	0	1.000	1.000	1.000
Malua Bay, NSW	i	35.792	16	10	20	31	.615	.392	.468
Moruya, NSW	i	35.910	11	2	12	1	.846	.923	.885
Tuross Head, NSW	i	36.060	23	8	16	6	.742	.727	.736
Nageela Orchard (in), NSW	i	36.160	23	59	9	38	.280	.191	.248
Nageela Orchard (out), NSW	o	36.160	26	12	25	14	.684	.641	.662
Narooma, NSW	i	36.214	8	0	6	0	1.000	1.000	1.000
Fairhaven, NSW	o	36.389	1	180	1	190	.006	.005	.005
Cobargo, NSW	o	36.427	0	0	2	2	•	.500	.500
Bega, NSW	i	36.676	44	0	34	1	1.000	.971	.987
Tathra, NSW	i	36.730	7	3	1	2	.700	.333	.615
Merimbula, NSW	i	36.889	12	0	7	1	1.000	.875	.950
Pambula, NSW	o	36.931	5	16	6	11	.238	.353	.289
Eden, NSW	i	37.065	33	8	15	2	.805	.882	.828
Nicholson River, Vic	o	37.796	29	79	30	60	.269	.333	.298
Johnsonville, Vic	i	37.816	27	2	29	2	.931	.935	.933
Bunyip, Vic	i	38.076	21	1	15	1	.955	.938	.947
Grovedale, Vic	i/o	38.207	3	1	0	2	.750	.000	.500
Lorne, Vic	o	38.534	5	11	6	2	.313	.750	.458
Rosevear, Tas	i	41.350	3	87	•	•	.033	•	.033
Hillwood, Tas	i	41.244	30	3	29	3	.909	.906	.908
Grove, Tas	i	42.989	100	0	22	0	1.000	1.000	1.000
Trial Bay Orchards, Tas	i	43.138	44	1	27	4	.978	.871	.934
Cygnets, Tas	i	43.161	29	0	33	0	1.000	1.000	1.000

NSW: New South Wales; Vic: Victoria; Tas: Tasmania; in/out: inside a building or shelter (i), or outside (o), or mixed (i/o); °Slat: degrees south latitude. mel males: number of *D. melanogaster* males; sim males: number of *D. simulans* males; mel females: number of *D. melanogaster* females; sim females: number of *D. simulans* females; mel/males: proportion of *D. melanogaster* among males; mel/females: proportion of *D. melanogaster* among females; mel/both: proportion of *D. melanogaster* among both sexes (pooled).

sorted under a stereo microscope to discard species other than *D. melanogaster*. Sites with fewer flies received much more collection effort in order to collect reasonable numbers of flies, so the data cannot be used to infer latitudinal or other patterns of absolute abundances. Some collections were made at outdoor sites, whereas others were made inside open buildings or sheds. Since *D. simulans* is thought to not enter buildings, sites were documented as indoors (i) or outdoors (o) or a mix (i/o) to determine if the species' proportions differed between these categories.

Counts were kept of *D. melanogaster* and *D. simulans* male and female numbers. The males of *D. melanogaster* and *D. simulans* can be easily discriminated by the shape of the genital arch of the male (Coyne, 1983; Shorrocks, 1972). The females can usually be discriminated by the pattern of dark pigmentation on the sixth and seventh abdominal tergites (Thompson *et al.*, 1979; Eisses and Santos, 1997; A. Hoffmann, personal communication). Female flies whose tergites had pigment extending to the lateral edges were scored as *D. melanogaster*; those with the pigment band stopping before the posterior-lateral corner of the tergite were scored as *D. simulans*. All males were discarded after scoring. Individual *D. melanogaster* females were put into fresh food vials with a sprinkle of live baker's yeast, and allowed to lay eggs in order to establish isofemale lines; these lines were later checked to be certain that no *D. simulans* had been included by examining the genitalia of offspring males. Fewer than 5% of these lines were *D. simulans*, indicating that my ability to rapidly sort females was good (though not perfect).

Flies were very numerous at some localities, so the flies counted are only a sample from those populations. For expediency in the field, one sex was not scored from some collections; hence there are some missing data. The data are presented in Table 1.

Figure 1 shows the proportion of *D. melanogaster* determined from females plotted against that determined from males for each collection. As is apparent, there is a very good correlation between the two (Spearman Rank Correlation, Rho (corrected for ties) = .907), and the Wilcoxon Signed Rank test indicated that the proportions did not differ significantly between the two sexes (Z-value = -1.384, $P = .1664$). The data for the two sexes were thus combined. The three outliers (Tathra, Grovedale and Lorne) are based on very small samples ($n = 13, 6$ and 24 , respectively).

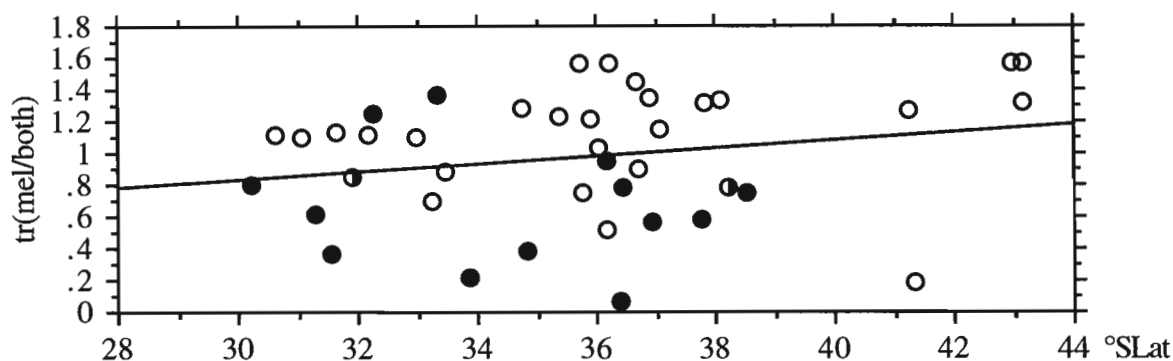


Figure 2. Regression of the proportion of *D. melanogaster* against latitude. Each site was scored as indoors (open circles), mixed (half-dark circles) or outdoors (dark circles). The ratios of pooled data for both sexes were arc-sine square-root transformed before analysis. The regression line has the formula $\text{tr}(\text{mel/both}) = 0.114 + 0.24 \times \text{°S lat}$, $r^2 = 0.04$. The ANOVA for the effect of the slope yields $F = 1.68$, $P = 0.203$.

Figure 2 shows the proportion of *D. melanogaster* (both sexes combined, arc-sine square-root transformed data) plotted against degrees south latitude. The unweighted regression analysis showed no significant clinal pattern over latitude in that proportion, although there was a slight (non-significant; $P = 0.203$) increase towards the south. Visual inspection of Figure 2 leads to the same conclusions: there was great variability between sites, but latitude was not a strong determinant of species proportion.

The data in Figure 2 are shown as indoors (open circles), mixed (half-dark circles) or outdoors (dark circles). A Kruskal-Wallis test of the significance of these groupings on the proportions of *D. melanogaster* yielded H (corrected for ties) = 11.53, $P = 0.003$, indicating that these three categories of sites differed

significantly in their proportions of the two species. While it is obviously not a strict rule, a higher proportion of *D. melanogaster* was found in indoor sites, and the reverse was true for *D. simulans*.

Acknowledgments: The Division of Entomology of the Commonwealth Scientific and Industrial Research Organization (Canberra, Australia) and the laboratory of Drs. John Oakeshott and Robyn Russell contributed significant logistic support to this project. This work was partly supported by a grant from the US Public Health Service (R01 GM49362).

References: Coyne, J.A., 1983, *Evolution* 37: 1101-1117; Eisses, K.T., and M. Santos 1997, *Dros. Inf. Serv.* 80: 87-89; Shorrocks, B., 1972, *Drosophila*. Ginn and Co., Ltd., London; Thompson, J.N., Jr., B.N. Hisey and R.C. Woodruff 1979, *Southwestern Naturalist* 24: 204-205.

The effect of *Drosophila* larvae on the pH of their resource.

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Introduction

A number of studies have examined the relationship between *Drosophila* performance and resource acidity. These include investigations into the success of *Drosophila* larvae (Burdick and Bell, 1954; Posch, 1971; Hodge *et al.*, 1996), developmental stability (Goldat and Beliaieva, 1935; Gordon and Sang, 1941), and the responses of adult *Drosophila* to acidic media (*e.g.*, Fluegel, 1981).

A point which is often overlooked is that the pH of the resource may change with time. Thus, the correlation of responses in *Drosophila* performance with the initial pH of the resource may be erroneous, as this is unrepresentative of the pH the animals actually encounter (see Hodge and Caslaw, 1998). This aspect of the system may have further significance as the changes in resource pH may be caused by the *Drosophila* themselves (Pearl and Penniman, 1926; Bridges and Darby, 1933); the common reference to *Drosophila* as 'vinegar flies' has long been testimony to their association with the acidification of fermenting substances (see Unwin, 1907). Interactions between *Drosophila* have sometimes been ascribed to modifications in the environment caused by larvae ('resource conditioning') (Weisbrot, 1966; Budnik and Brncic, 1975; Dolan and Robertson, 1975). Modification of resource pH is a potential mechanism via which the effects of conditioning may become manifest.

This paper describes the changes which occurred in the pH of *Drosophila* resources and established how these changes were affected by *Drosophila* larvae. The pH changes in artificial and natural resources were examined and the influence of the initial pH on subsequent pH modification was investigated.

Methods

General methods

Two species of wild-type *Drosophila* were used in this study: *D. melanogaster* ('Kaduna') Meigen and *D. hydei* Sturtevant. All experiments were carried out using standard glass vials (75mm × 25mm diameter), plugged with polyurethane foam bungs, as the experimental vessel. Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent) was used as the laboratory rearing resource. The pH of the resource was determined using an electronic pH meter [Jenway 3015, Jenway Ltd., Essex, England].

The effect of larval density on induced pH changes

Vials of resource were set up using 1.0g of IDM and 4.0ml of distilled water and the initial pH measured. Four replicates of seven densities of first instar *D. melanogaster* larvae (0, 2, 4, 8, 16, 32, 64) were then added to these vials. The pH of the resource was measured again when pupation of the larvae had ceased.