MATING CONTROL BY GENE ARRANGEMENTS IN *DROSOPHILA PSEUDOOBSCURA*[1]

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WITH a positive correlation between mating speed of homokaryotypes and the frequency of gene arrangements from the Mather, California, population of *Drosophila pseudoobscura*, it became clear that gene complexes included in those arrangements must play a prominent role in courtship behavior, a role essential to fitness in natural populations (SPIESS and LANGER 1964a). In its sibling species, *D. persimilis*, similar results had been obtained (SPIESS and LANGER 1964b). Further analysis of mating propensity demonstrated how extensively these features of behavior are controlled by gene arrangements in *D. pseudoobscura* (see reports by KAUL and PARSONS [1965, 1966] and EHRMAN [1965, 1966]). Several questions need to be answered, however, before any general statements can be made about the relationship between frequency and mating speed control in natural or artificial populations. For example, (1) Could the rare arrangements be accounted for on the basis of greater mating propensity in heterokaryotypes? (2) Did mating speed fit an "additive" model or were mating speeds unpredictable from the standpoint of homokaryotype performance? (3) Could it be determined which sex exerted greater control over mating speed?

Using the same strains and techniques of our earlier study (1964a) we extended the work to include heterogamic mating combinations. With five arrangements, 15 karyotypes, or 225 mating combinations are possible. With several strains of each arrangement and the intention to make all tests in duplicate, it became obvious that some economy would be necessary: we consequently omitted the rare heterokaryotypes TL/CH, TL/PP and CH/PP and restricted ourselves further to combinations in which only two arrangements were tested at a time (for example, no tests of AR/TL × ST/CH were made).

MATERIALS AND METHODS

Our techniques have been described in the papers cited. Briefly, 10 strains of AR, ST, TL, and PP and 7 strains of CH arrangements (third chromosome structural rearrangements: Arrowhead, Standard, Tree Line, Pikes Peak, and Chiricahua) were available, all descendants from Mather, California, population collected by DOBZHANSKY in 1959. Strains were intercrossed, and all flies tested were F_1 individuals cultured at 25°C. Ten strain-crosses were made for each combination which was tested in duplicate. Emerging adults were sexed and separated, then stored for six days at 15°C. In the later tests on aging and temperature for AR and PP, flies were cultured and stored differently (see below).

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TABLE 1

Example of average index in mating speed

Minutes	Weight	Number of matings	Maximum	Minimum
5	20	6	10	0
10	10	1	0	0
15	7	0	0	0
20	5	0	0	0
25	4	1	0	0
30	3	0	0	0
After 30	1	2	0	10
Mating Index:		136	200	10

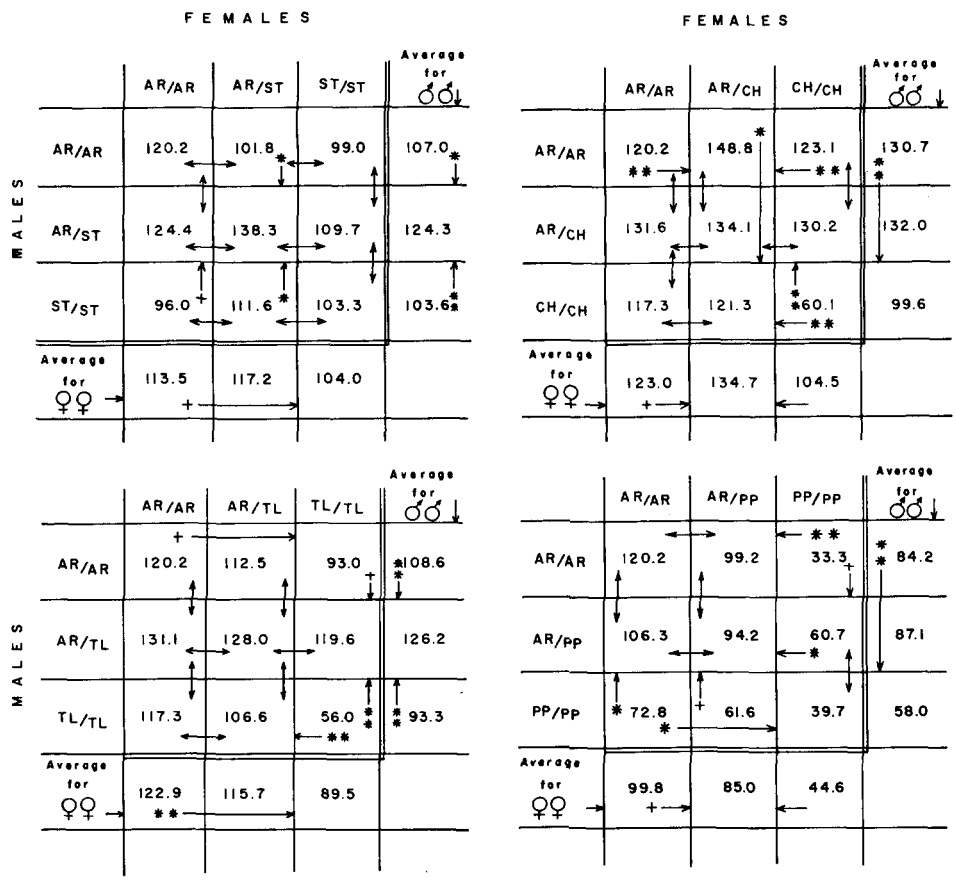

FIGURE 1.—Mating indices for karyotype combinations with Arrowhead arrangement. Upper left AR-ST, upper right AR-CH, lower left AR-TL, and lower right AR-PP. Average values are given in margins of each 3 × 3 table. Approximately, an index of 100 signifies 50% mating in the first 5 minutes. Arrows indicate probabilities for differences, horizontal = between females, vertical = between males. Double-ended arrows = nonsignificant F values, single-ended with a plus (+) = $5\% < P < 10\%$, single-ended with an asterisk (*) = $1\% < P < 5\%$, and those with double asterisk (**) $P < 1\%$. Arrows running through two "boxes" imply a contrast of two combinations *vs.* a third.

Twenty-four hours before mating tests, flies were counted in lots of ten per sex and transferred to freshly yeasted food vials. They were introduced into plastic mating chambers then without etherization, ten pairs per chamber at 25°C. When mating occurred, time was recorded and pairs in copulation were removed with an aspirator to prevent males being allowed a second mating. For each karyotype combination 200 pairs were tested. Homogamic mating data were used from the previous study (300 pairs per combination), but repeat tests were made to check on their reproduceability.

Previously, mating propensity has been reported as a cumulative percentage curve or as an average speed from a probit transformation plotted against log of time (SPIESS and LANGER 1964b), neither of which was satisfactory for statistical analysis. Since most of the percentage curves had the same shape owing to the fact that most matings, whether in fast or slow strains, tend to occur within the first ten minutes, it was found useful to weigh the number of matings per 5 minute intervals by the reciprocal of time × 100, plus a weight of 1 for those not mating after 30 minutes. An example of this mating index is given in Table 1. Such an index is useful

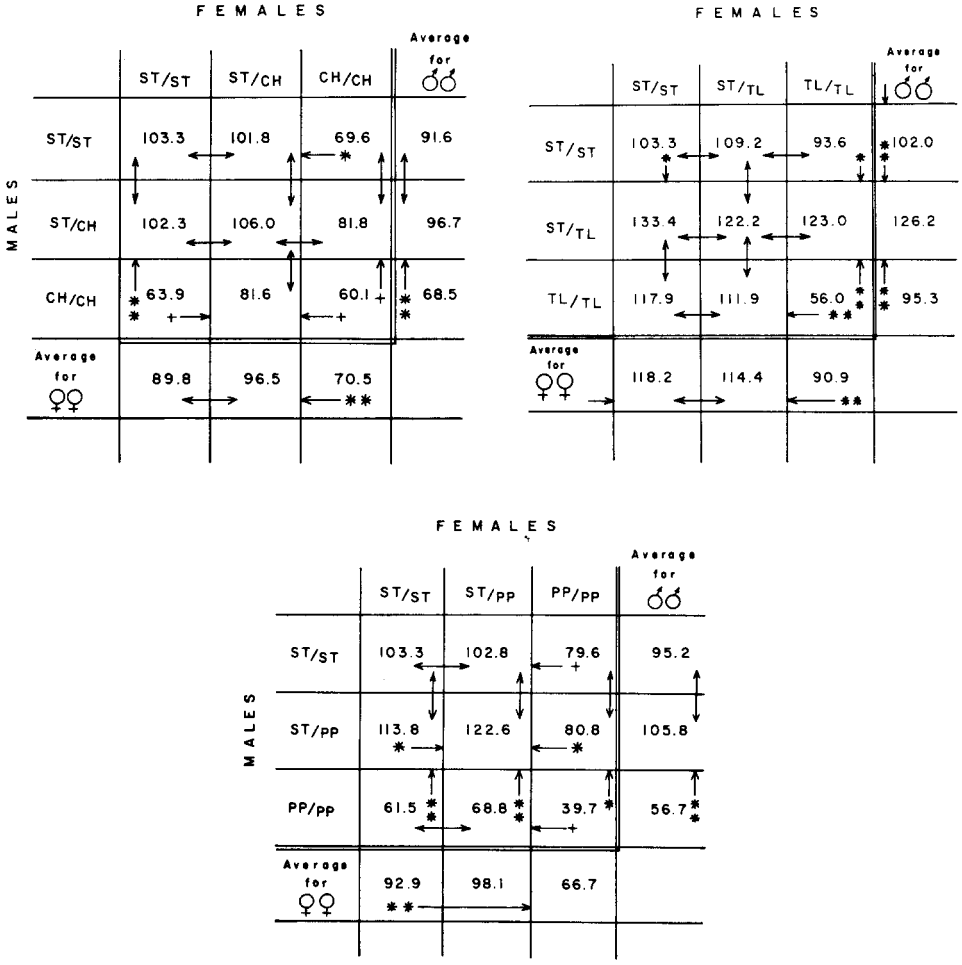

FIGURE 2.—Mating indices for karyotype combinations with Standard arrangement: upper left ST-CH, upper right ST-TL, and lower ST-PP.

in variance analysis, it avoids zeros, and gives an approximate comparative basis for the large number of karyotype combinations encountered.

Analyses of variance were computed on karyotype combinations with only two arrangements concerned at a time in 3 × 3 tables. Each duplicate pair was averaged to give ten indices for the karyotype mating combination. For the F test on marginal totals (average effects of either sex), the error was the mean square within combinations, assuming a fixed model for each karyotype, with 81 degrees of freedom, rather than the interaction mean square with only 4 degrees of freedom. Between any two combinations ("boxes") within the 3 × 3 table, there were 18 degrees of freedom for error and 1 for the difference.

RESULTS

The indices for karyotypes with AR *vs.* ST, *vs.* TL, *vs.* CH, and *vs.* PP are given in Figure 1, while Figure 2 indicates those of ST *vs.* TL, *vs.* CH, and *vs.* PP. The F test results are included in Table 2 for each pair of arrangements. Arrows in the figures indicate probabilities for differences, horizontal being between females and vertical between males. Double-ended arrows represent nonsignificant F values, single-ended with a plus (+) signify probabilities between 5% and 10%, single-ended with one asterisk (*) probabilities between 1% and 5%, and those with double asterisk (**) probabilities less than 1%. Arrows running "through boxes" imply a contrast of two combinations *vs.* a third. It should be noted that Table 2 does not include all the contrasts possible for the marginal totals; for the sake of brevity only the F values are given in each case, for (1) the contrast of homokaryotype (HOK) AR (or ST) *vs.* the heterokaryotype (HTK), and (2) the joint combinations of AR/- (or ST/-) *vs.* the rarer homokaryotype.

TABLE 2

Analyses of variance F values for pairs of gene arrangements and mean squares within karyotypes

Source	df	AR-ST	AR-TL	AR-CH	AR-PP	ST-TL	ST-CH	ST-PP
Between karyotypes	8	2.20*	6.09**	2.64*	8.53*	5.47**	2.64*	5.72**
Between males	2							
Contrast:								
(1) HOK AR(ST) *vs.* HTK	1	5.09*	5.38*	<error	<error	9.30**	<error	1.31
(2) AR-(ST-) *vs.* rarer HOK	1	3.29†	13.35**	27.48**	14.20**	7.55**	10.33**	30.29**
Between females	2							
Contrast:								
(1) HOK AR(ST) *vs.* HTK	1	<error	<error	2.80	3.05†	<error	<error	<error
(2) AR-(ST-) *vs.* rarer HOK	1	3.00†	20.35**	16.23**	42.58**	13.76**	8.09**	13.02**
Interaction	4	1.49	2.19†	4.94**	2.08†	3.23*	<error	<error
Within karyotypes (Mean Squares)	81	864	870	734	1074	942	1269	1269

†=.10 > P > .05. *=.05 > P > .01. **=P < .01.
HOK=Homokaryotype; HTK=Heterokaryotype.

The first contrast tests for heterotic change when the rarer arrangement is substituted, while the second contrast tests for lowering of mating index with the rarer homokaryotype.

Male comparisons: A consistently higher index is found among heterokaryotypes (vertical) throughout AR-ST, AR-TL, ST-TL, and ST-PP, which is reflected in significant F values (except ST-PP) in Table 2, upper contrasts. In AR-CH and ST-CH, two of the three columns show superior heterokaryotype males, so that in all there are 17 out of 21 columns with highest values for male heterokaryotypes. In every case the average effect of heterokaryotype males is highest although some are not significantly so (AR-CH, AR-PP, ST-CH and ST-PP). Nevertheless, if all average male data are combined and compared with AR/AR or ST/ST, the difference (upper half of Table 3) is highly significant (using the WILCOXON matched-pairs, signed ranks test; see SIEGEL, 1956).

Female comparisons: In contrast, there is no consistently higher value for female heterokaryotypes. In all rows there are 10 out of 21 with highest values for female heterokaryotypes though they are significantly so only in special cases (AR/CH × AR/AR and ST/PP × ST/PP), while average female indices show significant heterosis only for AR/CH. In the lower half of Table 3 it can be seen that there is no consistent difference between heterokaryotype females and AR/AR-ST/ST females, and in Table 2 none of the first contrasts for females is significant.

Reciprocal effects: It is worthwhile to make a comparison between the per-

TABLE 3

Average heterokaryotype vs. *average homokaryotype (Males: AR and ST only)*

Heterokaryotype (males)	Homokaryotype (males)	Difference
AR/ST	AR/AR	+ 17.3
AR/TL	AR/AR	+ 17.6
AR/CH	AR/AR	+ 1.3
AR/PP	AR/AR	+ 2.9
ST/TL	ST/ST	+ 24.2
ST/CH	ST/ST	+ 5.1
ST/PP	ST/ST	+ 10.6
		Average = 11.28 P = .01 *

Average heterokaryotype vs. *average homokaryotype (females)*

Heterokaryotype (females)	Homokaryotype (females)	Difference
AR/ST	AR/AR	+ 3.7
AR/TL	AR/AR	— 7.2
AR/CH	AR/AR	+ 11.7
AR/PP	AR/AR	— 14.8
ST/TL	ST/ST	— 3.8
ST/CH	ST/ST	+ 6.7
ST/PP	ST/ST	+ 5.2
		Average = 0.21†

* Using Wilcoxon matched-pairs, signed ranks test.
† Difference not significant.

TABLE 4

Reciprocals with heterokaryotype (HTK) males vs. *heterokaryotype females*

HTK	Mated to :	HTK males	HTK females	Difference
AR/ST	AR/AR	124.4	101.8	+ 22.6
	ST/ST	109.7	111.6	— 1.9
AR/TL	AR/AR	131.1	112.5	+ 18.6
	TL/TL	119.6	106.6	+ 13.0
AR/CH	AR/AR	131.6	148.8	— 17.2
	CH/CH	130.2	121.3	+ 8.9
AR/PP	AR/AR	106.3	99.2	+ 7.1
	PP/PP	60.7	61.6	— 0.9
ST/CH	ST/ST	102.3	101.8	+ 0.5
	CH/CH	81.8	81.6	+ 0.2
ST/PP	ST/ST	113.8	102.8	+ 11.0
	PP/PP	80.8	68.8	+ 12.0
ST/TL	ST/ST	133.4	109.2	+ 24.2
	TL/TL	123.0	111.9	+ 11.1
				Average = + 7.80
				.05 > P > .02*

* Using Wilcoxon matched-pairs, signed ranks test.

TABLE 5

Reciprocals with AR homokaryotypes

Mated to:	AR females	AR males	Difference
AR/ST	124.4	101.8	+ 22.6
AR/TL	131.1	112.5	+ 18.6
AR/CH	131.6	148.8	— 17.2
AR/PP	106.3	99.2	+ 7.1
ST/ST	96.0	99.0	— 3.0
TL/TL	117.3	93.0	+ 24.3
CH/CH	117.3	123.1	— 5.8
PP/PP	72.8	33.3	+ 39.5
			Average = + 10.8
			P = .15

Reciprocals with ST homokaryotypes

Mated to:	ST females	ST males	Difference
AR/ST	109.7	111.6	— 1.9
ST/TL	133.4	109.2	+ 24.2
ST/CH	102.3	101.8	+ 0.5
ST/PP	113.8	102.8	+ 11.0
AR/AR	99.0	96.0	+ 3.0
TL/TL	117.9	93.6	+ 24.3
CH/CH	63.9	69.6	— 5.7
PP/PP	61.5	79.6	— 18.1
			Average = + 4.7
			P > .20

formance of heterokaryotype males with heterokaryotype females in order to determine which is more effective in controlling the speed of mating. Table 4 shows a very nearly consistent higher value for males than females (the exception being the very high AR/CH female × AR/AR male index). Consequently heterokaryotype males seem to be more effective in determining the speed of mating. That this conclusion cannot be drawn for homokaryotypes of AR/AR and ST/ST is evident from Table 5: although the total difference favors the homokaryotype female in each case, the signed rank test indicates no significance in either.

Specific combinations: From Figures 1 and 2, it is quite obvious that mating speeds do not fit an "additive model." Not only is there considerable dominance and "overdominance" in the broad sense, so that heterokaryotypes do not conform to a linear, or additive, model, but marginal totals do not predict internal (within row or within column) effects in many cases. For example, AR/CH males average (132) a slight increment over AR/AR males (130.7) but when mated to AR/CH females mate less often (134.1) than AR/AR males (148.8). The significant interaction term in Table 2 expresses that special combining effect.

Secondly, it is important to notice that certain of the rarer arrangements combine well with either AR or ST while others are more specific: TL, for example, has very similar indices with both AR and ST, while CH does better with AR than with ST, but PP is reversed, that is higher with ST than with AR. In fact special combination interactions are perhaps more common than general predictable effects.

Finally, if only homogamic matings are compared, the heterokaryotypes (middle boxes in each 3 × 3 table) are higher than homokaryotypes (upper left and lower right) for all cases except the AR/PP × AR/PP which is intermediate. These were not indicated for statistical significance in the diagonal, but the consistency of the trend approaches significance ($P = 0.10$ with the matched-pair test).

Reproduceability of homokaryotype homogamic matings: Repeat tests of homokaryotype matings (SPIESS and LANGER 1964a) were made after all other experimental crosses were completed in April and May, 1966. The data are as follows (with standard errors of the mean for repeat data):

Mating karyotype	Original index	Repeat index	No.
AR × AR	120.2	113.4 ± 13.6	10
ST × ST	103.3	109.7 ± 8.1	20
TL × TL	56.0	72.6 ± 5.7	20
CH × CH	60.1	77.0 ± 11.3	20
PP × PP	39.7	33.4 ± 2.9	17

Repeating these matings demonstrates reasonable constancy after two years of maintaining the strains in the laboratory: their magnitude and relative order are very close. The TL × TL matings may have improved slightly, but none of the conclusions based on comparisons with other karyotypes are affected.

Aging and temperature effects on AR-PP: In their studies of mating speed,

PARSONS and KAUL (1966) did not find the very marked difference between AR and PP found in our laboratory. After a personal communication from DR. PARSONS, it was considered worthwhile to explore some possible causes for the discrepancy. While their strains come from the same collection as ours, only five strains of each arrangement were used by these authors. Nevertheless, if each arrangement has some general effect strong enough to be detected in mating behavior, we felt it essential to attempt to account for the different speeds in the two laboratories. PARSONS and KAUL had tested flies after 4 days and had raised them at two temperatures: 20° and 25°C. The performance of PP was equal to or better than AR, especially at cool temperature. Since all our flies were aged 6 days and all mated at 25°, we felt some accounting for the difference might be found in an aging temperature study.

Only homokaryotype AR and PP were used and only homogamic matings were made. All strain crosses were repeated as in the original experiments and matings were done in duplicate: flies were aged 2 days or 4 days and were raised and stored at either 15° or 25°C. Results are given in Figure 3. Note that at 2 days of age both AR × AR and PP × PP mating indices are lowest as expected. The temperature differences within arrangements are not significantly different at that age, while of course between arrangements AR is higher than PP. However at 4 days of age the situation is remarkably changed: the improvement in PP × PP

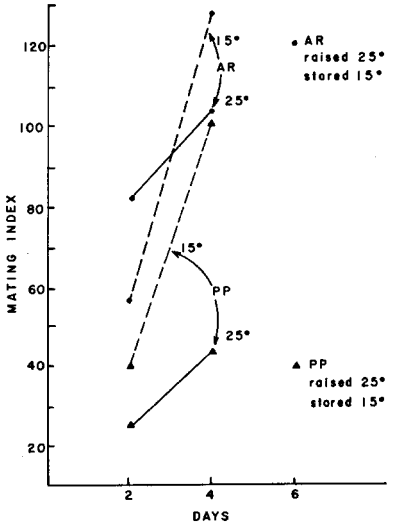

FIGURE 3.—Mating indices for AR × AR or PP × PP matings with flies aged two or four days and raised and stored at two temperatures: 25°C or 15°C. Dots = AR, triangles = PP; solid lines = 25°, dashed lines = 15°. Six-day old indices are given for comparison from the original data.

gained by raising and storing at 15° is much greater than the improvement gained by AR × AR; in fact PP at 15° is nearly identical with AR at 25°. (The points on the graph represented by the original data for 6 days of age are given for comparison.) Consequently it must be recognized that these mating speed tests are profoundly sensitive to environmental variables and aging.

DISCUSSION

It was suggested previously (SPIESS and LANGER 1964a) that balanced chromosomal polymorphism might be maintained if the observed differences in mating speed were characteristic of flies in the natural population from which they came. The less common arrangements in the Mather population (TL, CH, and PP) do have heterotic effects on male activity in mating speed at 25°C. Combining ability in heterokaryotype males is high between AR-TL and ST-TL, for example, and CH combines well with AR, while PP is better with ST. Without completion of all possible mating indices for the total 225 combinations in the population and calculating equilibria for the five gene arrangements on the basis of relative mating speeds, it is still reasonable from the combinations tested to assume that there can be an equilibrium maintained by this major component of fitness.

The determination of mating speed as a function of male activity is not only borne out by the average performances of males compared with females (that is, marginal indices in Figures 1 and 2) but also from reciprocal comparisons within the 3 × 3 tables. We are essentially in agreement with KAUL and PARSONS (1965) on this point, namely that the karyotype of the male was critical in mating speed determination.

Such importance for the male however is in contrast to the determination of mating speed by females in the sibling species, *D. persimilis* (SPIESS and LANGER 1964b; and also unpublished data on the Humboldt populations collected in 1964 —see SPIESS 1965). These observations confirm those made in sexual isolation studies between the species, in which it was found that *persimilis* males × *pseudoobscura* females produce more inseminations than the reciprocal cross. Rejection of *pseudoobscura* males by *persimilis* females is far more effective at preventing interspecific crossing than rejection by *pseudoobscura* females (for summary, see DOBZHANSKY 1951). If we can interpret mating as an interaction between the copulation tendency of males and the receptivity of females, the difference between these species might be generalized as the greater of the male tendency and nondiscriminatory female behavior in *pseudoobscura* with less male tendency and more female discriminatory behavior in *persimilis*.

The karyotypes and genotypes represented and the conditions utilized in our studies may not be characteristic of the average situation, since much larger samples and a wider range of conditions are needed before generalizations can be made for the entire species. The danger of such generalization is obvious when two laboratories report different mating speeds for similar material, as found by the studies of PARSONS and KAUL (1966) and of us on AR-PP arrangements. It is only fair to state that only five strains of each arrangement were used by

those authors, and in our laboratory the ten strains of PP were not uniformly low mating at 25°: two strains were consistently higher than the other eight, and if those alone had been used, there would perhaps have been no significant difference between AR and PP, just as PARSONS and KAUL reported. We agree that 25° is less favorable than lower temperatures (20° for those authors or 15° in our tests), and that PP matings are improved more than AR matings by the temperature change. Behavioral traits might be expected to be more sensitive to gene-environmental interactions than morphological or physiological traits, and the choice of measure can be very critical, as stressed by those authors; their discovery that heterokaryotypes vary less in performance between temperatures is noteworthy.

The amount of within-karyotype variation is due to differences between-strain crosses and replicates within-strain crosses. The latter are usually remarkably alike, and for the sake of brevity have been omitted from the analyses, but strain crosses were often consistently different from the average karyotype performance. This was particularly true in the AR-PP and ST-PP crosses, owing mostly to the two high PP values mentioned above. Based on the low mating propensity of most PP strains, we had some reasonable doubt that those exceptional faster mating strains might be indeed PP; salivary chromosome preparations were then made to check on the PP from those strains, and they were established as homokaryotype PP. Consequently it can only be assumed that considerable genetic variation must be available within strains and that the attainment of a particular mating speed index may be brought about by diverse developmental-genetic pathways. Nevertheless, the control of mating speed by the genetic complexes included in these chromosomal arrangements is significant, demonstrable, and informative about components of fitness in these populations, in spite of the clear need for standardization of techniques, measurement and experimental conditions.

SUMMARY

Mating speeds were determined for heterogamic combinations with heterokaryotypes and homokaryotypes of third-chromosome arrangements AR, ST, TL, CH, and PP from the Mather, California, population using the strains and techniques of SPIESS and LANGER (1964). The less common heterokaryotypes (TL/CH, TL/PP, and CH/PP) were omitted, and matings were restricted to combinations with only two arrangements at a time. An index of mating speed was computed by weighing the number of matings per 5-minute interval by the reciprocal of time × 100, plus a weight of 1 for those not mating after 30 minutes. —Male heterokaryotypes display a consistently higher index than homokaryotype males throughout, though some particular averages are not significantly higher. While certain heterokaryotype females are high (AR/CH), they do not show consistent superiority. In reciprocal effects for heterokaryotype performance, the speed of mating is significantly controlled by males.—Interactions are significant in many cases (that is, unpredictability from marginal totals), although none of the data can be considered "additive" since "overdominance" or

dominance is much more the rule. Special combinational effects are also found: CH combines better with AR than with ST, but PP is better with ST than AR; and TL combines with AR equally well as with ST. If only homogamic matings are compared, heterokaryotype × heterokaryotype is superior to homokaryotype in every case except AR/PP × AR/PP.—In a test of aging and temperature effects on mating speed with AR and PP only, all matings were better at 15° than at 25° and better at 4 days than at 2 days. However, the improvement in PP is far greater than in AR, so that 4 day-old PP at 15° mates equally well as 4 day-old AR at 25°. Mating-speed tests are thus very sensitive to environmental and aging variables.—These results are compared with those of PARSONS and KAUL (1966) for male activity, and possible explanations are offered for a discrepancy in AR-PP mating speeds.

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