Intrapopulational genetic variation in the hybridization between *Drosophila melanogaster* females and *D. simulans* males

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Summary. Intrapopulational variation on interspecific crossing ability between *D. melanogaster* and *D. simulans* has been measured. When the *melanogaster* females and *simulans* males were crossed, hybridization ranged from 3 to 34%, the female component of variation being more important than the male component. This point is discussed in relation with the role played by each sex in sexual isolation.

Key words. Drosophila melanogaster; Drosophila simulans; intrapopulational variation; interspecific crossing; hybridization.

Interspecific hybridization may be used as a measure of the level of genetic divergence between related species[1]. This enables us to infer phylogenetic relationships. Sexual isolation between *D. melanogaster* and *D. simulans* is not absolute, and in nature as well as in the laboratory many sterile hybrids can be produced. The interspecific hybridization is more frequent when the male is of *D. simulans* and the female of *D. melanogaster* than vice-versa[2]. Some relevant factors involved in the success of interspecific pairing in the laboratory are age, density, sex ratio, and specially, the genetic composition of strains employed, with mating values which ranged from 0 to 69%[3]. Nevertheless, there are no data concerning the intrapopulational variation in hybridization nor on the effect of captivity upon this character.

We used one population each of *D. melanogaster* and *D. simulans* captured in the same location in Asturias, Spain. Thus, they are sympatric. The populations were kept as isofemale lines at room temperature. All the experiments were carried out with 6-h-old adult flies whereby five individuals of each sex and species were introduced into a culture vial with standard food. Five days later, the females were individually assessed for progeny production. Because previous results had shown that the frequency of hybridization of *simulans* females was close to zero, we made only a small 5 × 5 isofemale line experiment of crossings *D. melanogaster* males to *D. simulans* females. This was done at the third laboratory generation. None of the 125 females of *D. simulans* produced progeny.

In the reciprocal cross direction, two experiments were run. In experiment I, an analysis of 16 × 16 isofemale lines of *D. melanogaster* females with *D. simulans* males was carried out at the third laboratory generation. Two replicates were made for each combination. In this way, a total of 2560 females of *D. melanogaster* were analyzed. Experiment II was carried out a year later after 14 laboratory generations. Some isofemale lines were accidentally lost and therefore the analysis was constructed with 13 lines of *D. melanogaster* and 12 lines of *D. simulans* only, giving a total of 1560 females to be examined. In all cases, the percentage of hybridization per vial was the number of females that produced hybrid progeny out of five.

Raw data appear in tables 1 and 2. The percentages were transformed by the arc-sine function, corrected for small size as suggested by Snedecor and Cochran[4] and submitted to an analysis of variance. Table 3 shows the observed mean squares. The components of variation are given as percentages. There exists an important genetic component in the determination of hybridization. This genetic component is mainly of male × female interaction but the female component is more important than the male component of variation.

The results of experiment II, made a year later, are given in table 3. The mean square (MS) of error has not changed and neither has the percentage of genetic component; yet the absence of interaction between the sexes is noteworthy. This last result suggests the existence of a genotype-experiment interaction, which could be related with the adaptation of the flies to the laboratory environment during captivity. Again, the female component of variation is higher than the male component. A similar result was found by Parsons[5], although he worked on intraspecific variation whereas our experiments deal with intrapopulational variation.

Table 1. Mean percentages of hybridization observed in experiment I, between males of *D. simulans* and females of *D. melanogaster*, from different isofemale lines

Lines of *D. melanogaster* (females)	Lines of *D. simulans* (males)															
	30	10	0	20	10	0	20	10	20	0	10	0	20	20	40	10
	10	0	30	0	10	10	30	30	10	0	10	10	20	20	70	0
	30	0	10	10	0	10	10	20	10	10	30	10	30	0	20	10
	0	10	30	10	10	40	30	10	10	10	10	0	30	10	0	0
	0	0	20	20	70	50	10	20	40	0	10	30	0	0	0	10
	40	60	60	30	50	30	20	20	10	30	20	0	30	20	40	50
	10	0	10	10	20	10	20	0	10	10	0	0	40	0	10	0
	30	0	10	40	40	30	30	10	0	0	0	10	40	20	20	10
	50	0	0	10	20	10	10	10	10	20	0	20	20	40	30	50
	0	20	20	40	0	10	20	10	0	20	0	0	50	0	0	10
	50	60	40	20	50	50	20	0	20	30	20	0	60	0	10	20
	30	20	0	60	40	30	0	20	10	30	40	40	40	10	20	0
	10	50	20	0	10	10	10	0	10	60	0	40	60	50	10	0
	70	70	70	20	10	60	20	0	10	20	0	20	40	30	0	20
	60	80	20	20	50	30	0	20	40	10	40	20	20	20	10	10
	0	10	10	10	0	0	50	0	20	20	10	20	10	10	0	0

The population mean, and the mean of each isofemale line, in angles, appear in table 4. Because the variances of males and females between experiments I and II are different, we used the method described in[4] to compare the population means I and II in each sex. Significant differences were not found. Thus, the populations means of neither sex changed. However, we observed a certain trend towards a diminution of hybridization ability during captivity, as is shown by the significant differences found among the mean values of some isofemale lines between experiments I and II (table 3), when they were compared by a Student's t-test. The increase of variance between lines observed in experiment II when contrasted with experiment I, would be explained by the increase of endogamy during the 14 laboratory generations, as well as the dispersive process inherent to the small population size of the isofemale lines[6].

In *Drosophila* there are contrasting hypotheses which confer an essential role to the sexual behavior of males[7] or females[8] in relation with premating mechanisms of sexual isolation. The evidence from laboratory data is not conclusive since it is dependent on the ethological factors that are generally considered important. In the pair of the sibling species *melanogaster-simulans*, it appears that the male behavior during the first phases of courtship is essential in interspecific hybridization, the *simulans* male showing a more specific behavior than the less selective *melanogaster* male[9]. However, this is in clear contrast with the results of this paper and the general observations on hybridization, which point out that the break of interspecific isolation occurs more through the male of *D. simulans* than the male of *D. melanogaster*. Manning[10] found in the same species that when the adults are young the female has a fundamental role in sexual isolation while both sexes have similar importance when they aged. Our results, using very young adults, support this point since the higher genetic component for *melanogaster* females compared to *simulans* males suggests that the former play a major role in reproductive specific isolation. This conclusion is not surprising since in homospecific pairing the copulation is mainly dependent on female acceptance[11].

Table 2. Mean percentages of hybridization observed in experiment II, between males of *D. simulans* and females of *D. melanogaster*, from different isofemale lines

Lines of *D. melanogaster* (females) \ Lines of *D. simulans* (males)												
	0	0	0	0	0	10	0	0	20	10	0	10
	50	0	10	10	0	20	0	0	20	10	0	20
	10	0	0	0	10	0	0	0	10	10	0	10
	70	0	10	20	10	30	20	0	40	0	0	30
	70	10	50	0	40	70	70	40	70	0	10	40
	20	40	0	10	0	20	20	20	40	0	40	40
	0	10	30	30	10	0	10	30	30	10	10	20
	70	0	50	30	40	40	50	0	50	30	20	80
	0	0	30	0	10	10	10	10	10	0	0	0
	40	10	60	0	20	30	30	20	20	0	30	30
	80	10	10	0	0	20	20	0	10	20	30	10
	60	0	0	10	20	40	20	10	40	0	0	0
	40	10	10	20	20	0	10	0	10	0	0	40

Table 3. Analysis of variance of the percentages of hybridization between *D. melanogaster* females and *D. simulans* males, in experiments I and II. The data have been transformed by the arcsin function. The F-values of males and females in experiment I were obtained using the mean squares (MS) of interaction

	Source of variation	D.F.	M.S.	F	P	Components of variation
Experiment I	Males	15	454.37	1.96	< 0.05	3.44%
	Females	15	671.76	2.90	< 0.001	6.68%
	Interaction	225	230.90	1.72	< 0.05	28.81%
	Error	256	134.09			65.97%
Experiment II	Males	11	1425.77	6.46	< 0.001	14.19%
	Females	12	1654.37	7.50	< 0.001	18.29%
	Interaction	132	273.51	1.24	n.s.	–
	Error	156	220.55			67.52%

Table 4. Population means (± SE) and means of each isofemale line, of the percentage of hybridization of *D. melanogaster* (females) and *D. simulans* (males), found in experiments I and II, along with the significance of the differences between them. All the values are in the angular scale

D. melanogaster			*D. simulans*		
Experiment I	Experiment II	Difference	Experiment I	Experiment II	Difference
19.19	23.89	n.s.	20.50	lost	–
20.01	17.87	n.s.	21.07	14.81	n.s.
21.19	12.34	< 0.05	21.16	26.62	n.s.
21.65	21.38	n.s.	22.02	lost	–
21.72	10,08	< 0.05	22.47	23.68	n.s.
22.15	10.08	< 0.05	23.33	12.63	< 0.05
23,66	16.14	n.s.	23.99	22.67	n.s.
24.29	lost	–	24.45	15.34	< 0.05
24.88	19.75	n.s.	25.27	lost	–
25,34	35,66	< 0.05	26,15	14,85	< 0.05
26.75	lost	–	27.31	22.31	n.s.
28.75	20.81	< 0.05	28.46	lost	–
31.19	26.00	n.s.	28,60	12.28	< 0.05
31.28	lost	–	28,79	18.23	< 0.05
31.52	19.85	< 0.05	29.94	35.67	n.s.
33.65	36,14	n.s.	33,72	30.14	n.s.
25.45 ± 1.14 (15 d.f.)	20.77 ± 2.21 (12 d.f.)		25.45 ± 1.08 (15 d.f.)	20.77 ± 2.22 (11 d.f.)	

From our data we cannot determine whether the variation described is due to a variation in female receptivity and male virility of *D. melanogaster* and *D. simulans* respectively, or whether it is related to variation in the sexual discriminative specific behavior, that is, sexual isolation. This problem is at present under study.

1 Spieth, H.T., Evol. Biol. *2* (1968) 157; Kaneshiro, K.Y., Evolution *30* (1976) 740; Watanabe, T.K., and Kawanishi, M., Science *205* (1979) 906.
2 Sturtevant, A.H., Genetics *5* (1919) 488.
3 Watanabe, T.K., personal communication.
4 Snedecor, G.W., and Cochran, W.G., in: Statistical Methods. Iowa University Press, Ames, 1967.
5 Parsons, P.A., Can. J. Genet. Cytol. *14* (1972) 77.
6 Falconer, D.S., in: Introduction to Quantitative Genetics. Oliver and Boyd, Edinburgh and London 1960.
7 Spieth, H.T., Bull. Am. Mus. nat. Hist. *99* (1952) 395; Spieth, H.T., A. Rev. Ent. *19* (1974) 385.
8 Bateman, A.J., Evolution *3* (1949) 174: Merrell, D.J., Genetics *34* (1949) 370.
9 Wood, D., and Ringo, J.M., Evolution *34* (1980) 320.
10 Manning, A., Anim. Behav. *7* (1959) 60.
11 Petit, C., and Ehrman, L., Evol. Biol. *2* (1968) 157; Spiess, E.B., in: Essays in Evolution and Genetics in honor of Th. Dobzhansky. North Holland Publ., Amsterdam 1970; Spieth, H.T., A. Rev. Ent. *19* (1974) 385.

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Comparison of the in vitro development of mouse single blastomeres with and without the zona pellucida

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Summary. No difference was observed during in vitro development between mouse single blastomeres with and without the zona pellucida, isolated from 2- and 4-cell stage eggs.
Key words. Mouse blastomeres; blastomeres, mouse; zona pellucida.

The developmental potential of individual blastomeres from cleaving eggs has been extensively studied in mammals. Two major methods have been employed for obtaining blastomeres; the destruction of sister blastomeres inside the zona pellucida[1–4], or isolation from each other after removal of the zona[5,6]. Each method has its advantages and disadvantages. The latter gives rise to naked blastomeres. The zona pellucida is necessary for the transit of the cleaving-stage egg through the oviduct in normal pregnancy[7,8]. However, the role of the zona in the development of blastomeres in vitro is little understood. We have recently developed an miproved method[9] for isolating mouse blastomeres by electrically destroying other blastomeres inside the zona. The present report describes the in vitro development of single mouse blastomeres with and without the zona pellucida.

Materials and methods. Cleaving eggs for preparation of single blastomeres were collected by flushing oviducts excised from C57Bl/crSlc females, which were injected with 5–10 i.u. of pregnant mare serum gonadotropin followed by the same dose of human chorionic gonadotropin (HCG) 48 h later and were mated with fertile DBA/2Slc males. Two-cell stage eggs were collected at 38–40 h, and 4-cell stage eggs at 48–50 h after the HCG injection. Single blastomeres with the zona were obtained by electrically destroying all blastomeres except one inside the zona as follows. The eggs were placed in a drop of the culture medium[10] under paraffin oil in a special dish[9]. While the egg was held by suction on the tip of the holding pipet connected to a micromanipulator, a glass microelectrode was gently pushed through the zona and was introduced into the unwanted blastomere, which was then charged by a direct current (60 μA, 8 V). The target blastomere disintegrated completely in a few seconds after the charge. Micromanipulation was carried out at room temperature and at a magnification of 200× under Nomarski optics.

Blastomeres without the zona were isolated as follows; the zona was removed by a 20–50-sec incubation with warmed (37 °C) acidic Tyrode's solution (pH 2.0)[11] and the zona-free embryo was separated mechanically into individual blastomeres with a fine-bore glass pipet in phosphate-buffered saline solution containing 0.02% EDTA and 0.2% trypsin. The blastomeres that were isolated by the two methods were incubated in drops of culture medium covered with paraffin oil at 37 °C in an atmosphere of 5% CO_2, 95% air.

Results and discussion. The table shows the in vitro development of various mouse embryos with and without the zona pellucida. The naked one-half embryos (½-embryos) isolated

In vitro development of single mouse blastomeres with and without the zona pellucida isolated from 2- and 4-cell stage eggs

With or without the zona	Sorts of embryos	No. of embryos	Embryonic structures at blastocyst stage: Blastocyst	Morula	Trophoblastic vesicle	Non-integrated form	Degenerated or arrested
With the zona	2-cell egg	62	59 (95.2)				3 (4.8)
	4-cell egg	66	61 (92.4)				5 (8.6)
	½-embryo	104	88 (84.6)	2 (1.9)	3 (2.9)	2 (1.9)	9 (8.7)
	¼-embryo	116	41 (35.3)	21 (18.1)	24 (20.7)	16 (13.8)	14 (12.1)
Without the zona	2-cell egg	78	71 (91.0)			3 (3.8)	4 (5.1)
	4-cell egg	67	65 (97.0)				2 (3.0)
	½-embryo	205	180 (87.8)	7 (3.4)	5 (2.4)	1 (0.5)	18 (8.8)
	¼-embryo	87	37 (42.5)		27 (31.0)	9 (10.3)	7 (8.1)

Values were obtained from 4 to 6 experiments. Values in parentheses are percentages of the number of embryos used.