Hence, a direct relationship exists between NOR number in mitotic chromosomes and the number of nucleoli in the spermatocytes of these species. As in *M. elegans*, 1 *D. australis* nucleolus was found to show a persistent association with the XY bivalent, being either contiguous (figure 3) or very close (figure 4). Serial sections in both species of spermatocyte nuclei[3,11] showed that the nucleoli are segregated and that the FC is intimately associated with lateral elements of the SC and with the nucleolar material (figure 5,b). The SC crosses through the FC and can be followed up to its insertion on the nuclear membrane (figure 5,a). As previously observed in spermatocyte nuclei of *M. elegans*, the SC that traverses the FC and extends from the nuclear membrane to the centromere, corresponds to the short arms of the bivalent formed by the chromosome C_2[11]. Using the same markers, 2 SC segments of different lengths have been found in *D. australis*. These segments are equivalent to the short arms of the C_1 and C_2 pairs in this species[3], as judged by the segment lengths of the SC involved and by the position of the markers (figure 6). It appears therefore that the sequence of telomere-NOR-centromere of the short arms of the mitotic chromosome corresponds to the proposed sequence in this work for meiotic chromosomes: insertion on the nuclear membrane-FC-centromere, along the SC. During pachytene, it is known[12] that paired bivalents are inserted on the nuclear membrane by their telomeric ends (figure 4) and that the SC extends along the whole length of the chromosomal pair[14]. If the NOR position in the mitotic chromosomes of these species is subterminal, as shown by the Ag-AS technique, then a similar position for the NOR is to be expected in meiotic chromosomes. Our observations show that the FC position on the SC is also subterminal. If we consider: a) the relationships between the FC with SC and with the nucleolar material[3,9,11,12], b) the ultrastructural resemblance of the FC to secondary constrictions as revealed by electron microscopy of mitotic chromosomes[17,18], and c) the other morphological data presented in this work, we are lead to conclude that the FC of the spermatocyte nuclei herein discussed, corresponds to the NOR zone. The nucleolar morphology is highly variable and its organization depends upon the stage in the cell cycle[2] as well as on the rRNA synthetic activity that takes place in this nuclear organelle[6,13]. In both species, the spermatocyte nucleolus displays a similar structure and organization. It has an irregularly elongated comma shape, with a thickened tip that includes the NOR, the fibrillar part and a fraction of the granular part; and a caudal end composed mainly by trabecules of the granular part. The nucleolus is associated with the SC by its thickened end, whereas its caudal one penetrates deep into the nucleus, approximately following the pathway of the nucleolar SC. Obviously, in order to ascertain the true position of the nucleolus within the nucleus, the NOR position should be considered. As the position of the nucleolus in spermatocyte nuclei depends upon the position of the NOR[2,3], and as the latter is adjacent to the telomere inserted on the nuclear membrane, it follows that the position of the nucleolus must be peripheral and close to the nuclear envelope in both species. It is probable that the above criteria, and rationale of nucleolus location in spermatocyte nuclei of *M. elegans* and *D. australis*, may also be valid for the same cell types in other animal species and for plant meiocytes.

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2 G. Giménez-Martín, C. de la Torre, J.F. López-Sáez and P. Esponda, Cytobiologie *14*, 421 (1977).
3 R. Fernández-Donoso, M.S. Berríos and J. Pincheira, Rev. Micr. Elect. *5*, 302 (1978).
4 W. Bernhard, C. Frayssinet, C. Lafarge and E. Le Buton, C.r. hebd. Séanc. Acad. Sci., Paris *261*, 1785 (1965).
5 E.G. Jordan and J.M. Chapman, J. exp. Bot. *22*, 627 (1971).
6 G. Goessens, Cell Tissue Res. *173*, 315 (1976).
7 G. Giménez-Martín and J.C. Stockert, Z. Zellforsch. mikrosk. Anat. *107*, 551 (1970).
8 P. Esponda and G. Giménez-Martín, Chromosoma *52*, 73 (1975).
9 E.G. Jordan and B.T. Luck, J. Cell Sci. *22*, 75 (1976).
10 L. Tres, Chromosoma *53*, 141 (1975).
11 R. Fernández-Donoso and R. Wettstein, Rev. Micr. Elect. *3*, 52 (1976).
12 C.B. Gillies, Chromosoma *43*, 145 (1973).
13 G. Goessens, Proc. 5th Eur. Nucleolar Workshop, Salamanca-Spain, 1977, p. 17.
14 R. Wettstein and J.R. Sotelo, J. Microsc. *6*, 557 (1967).
15 C. Goodpasture and S.E. Bloom, Chromosoma *53*, 37 (1975).
16 T.C. Hsu, S.E. Spirito and M.J. Pardue, Chromosoma *53*, 25 (1975).
17 T.C. Hsu, B.R. Brinkley and F.E. Arrighi, Chromosoma *23*, 137 (1967).
18 J.C. Lafontaine, Ultrastructure in Biological Systems vol. *3*, 152. Academic Press, New York and London 1968.

Sexual selection, *Drosophila* age and experience

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Summary. Drosophila females modify their choice of mates after an initial mating experience. The altered choices correspond to selective pressures within strains *(D. pseudoobscura)*, semispecies *(D. paulistorum)*, and full species *(D. melanogaster* and *D. simulans)* and indicate a learned component in sexual selection.

Drosophila females are known to mate repeatedly both in the laboratory and in the field[1]. We have shown that prior mating experience leads to altered choice of mates in subsequent multiple choice experiments[2]. *Drosophila pseudoobscura* Arrowhead females, for example, show a statistically significant preference for orange-eyed Standard males (autosomal recessive, or; Arrowhead and Standard represent different karyotypes) after an initial mating experience with males of this karyotype. We have further shown that this change in preference resembles learning in that it is subject to disruption by cyclohexamide, a protein synthesis inhibitor, in ways analogous to those reported for mice and

Table 1. Proportion of homogamic *Drosophila* matings as a function of age and prior mating experience. N = total number of observed matings, P = proportion of homogamic matings = $\frac{({}^{n}A♀ \times A♂ + {}^{n}B♀ \times B♂)}{{}^{n}A♀ + {}^{n}B♀}$

Cross	Number of subjects	3-day-old virgins	9- or 11-day-old virgins	9- or 11-day-old homogamic experience	9- or 11-day-old heterogamic experience
mel × sim	491	0.968	0.991	0.852	0.587
M × SM	329	0.875	0.875	1.000	0.833
CR × S	422	0.592	0.593	0.737	0.585
B × T	394	0.783	0.692	0.831	0.651
L1 × O	446	0.633	0.442	0.806	0.670

mel = *D. melanogaster;* sim = *D. simulans;* M = Mesitas, Colombia, Andean, *D. paulistorum* semispecies; SM = Santa Marta, Colombia, Transitional semispecies; CR = Costa Rica, Centroamerican species; S = Salvador, Centroamerican semispecies; B = Belem, Brazil, Amazonian semispecies; T = Tame, Colombia, Amazonian semispecies; L1 = Llanos, Colombia, Interior semispecies; and O = Georgetown, Guyana, Orinocan semispecies.

Table 2. t-test of differences between proportions of homogamic *Drosophila* matings. $t = \frac{P_1 - P_2}{S}$, where $S = \sqrt{pq\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$

Cross	3-day virgins vs 9-day virgins	9-day virgins vs homogamic experience	9-day virgins vs heterogamic experience	Homogamic experiences vs heterogamic experiences
mel × sim	−1.230	3.949	7.566	4.102
N	135	321	157	256
P	NS	< 0.001	< 0.001	< 0.001
M × SM	0.0	−3.102	0.494	3.494
N	240	191	138	89
P	NS	< 0.01	< NS	< 0.001
CR × S	−0.015	−2.019	0.122	2.159
N	228	1.84	226	194
P	NS	< 0.05	NS	< 0.05
B × T	1.602	−2.125	0.613	2.521
N	240	191	203	154
P	NS	< 0.05	NS	< 0.05
L1 × O	2.966	−5.557	−3.408	2.219
N	240	223	223	206
P	< 0.01	< 0.001	< 0.001	< 0.05

For legend see table 1.

fish[3]. Here we examine the effects, if any, of previous copulatory experience and concurrently, of aging, on ethological isolation between members of incipient *D. paulistorum* species as well as between sibling species *D. melanogaster* (mel) and *D. simulans* (sim).

Six incipient species or semispecies compose the *D. paulistorum* species-complex[4]. Among them intense reproductive isolation exists in assorted forms and with varying genetic architectures. 2 types are of interest here: 1. Sexual isolation - making matings within semispecies much more likely to occur than between semispecies. This behavioral isolation is fostered by polygenes distributed all over the 3 pairs of chromosomes possessed by this superspecies; and 2. Hybrid male sterility - occurring only as a result of forced intersemispecific crosses; this thoroughgoing (even into backcrosses) sterility is related to maternal genotypes. If it is a hybrid one, sons are sterile. For example, the crosses in either direction of Mesitas (M) × Santa Marta (SM) and of Llanos (Ll) × Orinocan (O) outlined in table 1 produce wholly sterile male progeny. Belem (B) × Tame (T) plus its reciprocal cross and Costa Rica (CR) × Salvador (S) plus its reciprocal are interfertile. The *D. paulistorum* species-complex is a relatively rare but precious example of species in statu nascendi.

The sibling species *D. melanogaster* and *D. simulans* rarely hybridize in nature, but mate with difficulty and have offspring under laboratory conditions. Factors known to influence their isolation include mixed culturing during metamorphic stages[5], age[6], the number and ratios of flies in a mating chamber and genetic backgrounds primarily from the maternal side[7]. Both *D. melanogaster* and *D. simulans* were wild-type, laboratory strains. The former is a stock begun with a single gravid female captured at Ceres, New York, and described previously[8].

Females were aged either 3 or 9 days for *D. paulistorum* and 3 or 11 days for *D. melanogaster* and *D. simulans;* all males were 3-4 days old. For copulatory experience, 3-day-old females were confined with either homogamic or heterogamic males for 1 day in mass cultures. Each female was then placed alone in a vial containing food for 5 days *(D. paulistorum)* or 7 days *(D. melanogaster* and *D. simulans).* Larvae indicated that mating had taken place and only females that produced larvae were used for further tests. Unanaesthetized flies were placed into chambers and all matings were scored by direct observation with a hand lens[9].

This method allows the simultaneous scoring of 4 possible types of matings, 2 homo- and 2 heterogamic: A♂ × A♀, B♂ × B♀, A♂ × B♀ and B♂ × A♀.

The proportion of homogamic matings for each condition and a t-test comparison[10] between conditions within a cross are presented in tables 1 and 2 respectively. With 1 exception, mating choices made by young females did not differ significantly from those of older virgin females. 3-day-old

females of Ll × O cross, however, showed a significantly higher bias towards homogamic males than did 9-day-old virgins.

Prior copulatory experience affected subsequent mating choices made by *Drosophila* females; the direction of the change depended on whether the crosses were within a species, between semi species or between sibling species. We showed previously[1-3] that following a heterogamic copulatory experience *D. pseudoobscura* females showed a strong bias towards males of the same karyotype as their initial mate. *D. paulistorum* females which mated with a homogamic male showed an increased tendency to remate with the same type males. Comparison between proportion of homogamic matings of sexually experienced and virgin 9-day-old females were statistically significant for all crosses tested. No effect of this magnitude due to initial heterogamic mating experience was observed. *D. melanogaster* and *D. simulans* females, however, showed a significant reduction in ethological isolation after both homo- and heterogamic initial copulatory experience, and in fact, choices made by females with heterogamic mating experience did not differ significantly from random mating. When *D. pseudoobscura* is similarly pitted versus its sibling species *D. persimilis*, Spieth found that each of these closely related species responds differently[11]. The females of both species will immediately accept their own male as soon as they are able to, even though under duress they had accepted the male of the other species and a *D. persimilis* female (like *D. paulistorum*), once she has been inseminated by a *D. persimilis* male, will never again accept a *D. pseudoobscura* male. The *D. pseudoobscura* female is never so restricted and is simply always less sexually isolated from potential *D. persimilis* mates (as *D. melanogaster* - *D. simulans*).

It is not immediately apparent why an initial 'no choice' mating experience had such diverse effects on subsequent choices of mates in these drosophilids. One may speculate that within a species, i.e., with *D. pseudoobscura*, selective pressures exist to enhance outbreeding via heterogamy, and at least an initial mating with a less frequent male will serve to increase the frequency of his karyo- or genotype in the population. For species in statu nascendi however, such pressures must be directed toward the avoidance of less fertile heterogamic matings.

Drosophila mating behavior has been viewed as primarily genetically determined and barely modifiable by experiences during development[12]. The results of these studies show, however, that previous copulatory experiences alter subsequent choice of mates and indicate that sexual isolation contains an acquired component.

1 A. Pruzan, Evolution *30*, 130 (1976).
2 L. Ehrman and J. Probber, Am. Sci. *66*, 216 (1978); E. O'Hara, A. Pruzan and L. Ehrman, Proc. natl Acad. Sci. USA, *73*, 975 (1976).
3 A. Pruzan, P.B. Applewhite and M.J. Bucci, Pharmac. Biochem. Behav. *6*, 355 (1977).
4 T. Dobzhansky and J.R. Powell, in: Handbook of Genetics, vol. 3, p. 537. Ed. R.C. King. Plenum, New York 1975.
5 M. Eoff, Am. Nat. *107*, 247 (1973).
6 J.S.F. Barker, Am. Nat. *101*, 277 (1967).
7 A. Manning, Viewpoints Biol. *4*, 125 (1967).
8 L. Ehrman, Am. Nat. *105*, 479 (1971).
9 L. Ehrman, Evolution *19*, 459 (1965).
10 A. Romano, in: Applied Statistics for Science and Industry. Allyn and Bacon, Boston 1977.
11 H.T. Spieth in: Behavior and Evolution, p. 363. Ed. A. Roe and G.G. Simpson. Yale Univ. Press, New Haven 1958.
12 P.A. Parsons, Adv. Genet *19*, 1 (1977).

Regional and strain variation in brain 3′:5′ cyclic adenosine monophosphate of inbred mice

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Summary. Regional variations were found in cAMP levels in flash frozen mouse brains with the pons and cerebellum having higher levels than the cerebrum. There were also strain variations with CBA/J and BALB/cJ having higher levels than C57B1/6J in the pons and cerebellum.

It is generally accepted that the binding of neurotransmitter stimulates cyclic AMP (cAMP) synthesis in some receiving cells[1]. There have been multiple attempts to study cAMP levels in brain samples[2,3] and several investigators have reported variations in brain cAMP levels among inbred strains of mice that may correlate with strain variations in behavioral parameters[4-6]. This work has neglected regional differences and indicated surprisingly high levels. In some of this work microwave irradiation was used to kill the mice and cAMP was measured for whole brain and expressed in terms of wet weight. This microwave method of killing the animal is not optimal[7]. We have re-investigated cAMP levels in several strains in a standardized method using liquid nitrogen to inactivate cAMP-degradative activity and have expanded the measurements to four regions of the brain.

Materials and methods. Male mice (6-week-old or older) were killed by decapitation such that the heads fell directly into 5 cm or more of liquid nitrogen. The procedure was standardized so that no more than 35 sec. elapsed from the moment the mouse was first disturbed until the brain was frozen solid (between 10 and 20 sec. elapsed from opening the cage until the head was submerged in liquid nitrogen). More important, in order to minimize the effects of ischemia[3], no more than 10 sec elapsed from the instant of decapitation until total inactivation of all neural enzymes[8]. The brains were then removed from the liquid nitrogen and immediately placed on dry ice and transferred to a cold room for dissection. Working in a cold room on dry ice, the head was bisected revealing a midsagittal view. 4 regional samples, about 1 × 3 mm and approximately 10 mg in wt of upper cerebrum, lower cerebrum, pons, and cerebellum of the brain were taken. Each frozen brain sample was placed in a small test tube for storage. 400 µl of warm (70 °C) dH_2O were pipetted into each tube containing the frozen tissue sample and immersed in a boiling water bath for 13 min. Samples were then removed and centrifuged for 20 min at 2400 × g at 4 °C. 100 µl of each supernatant (in