C. septemvittatus and the absence of a second 'a' allele at the SOD locus that is fixed in both *C. gularis* and *C. inornatus* implicate *C. septemvittatus* as a second parental species. Thus, alleles at 6 loci favor the involvement of *C. septemvittatus* and there are no alleles in conflict with this hypothesis.

The array of alleles in the unassigned haploid genome is similar to those of the 4 remaining taxa (table). The absence of alleles in *C. exsanguis* that are fixed in *C. b. burti* (MPI-1) and *C. b. xanthanotus* (GPI) remove these taxa from primary contention. All alleles favor equally the involvement of *C. b. stictogrammus* and *C. costatus* and provide no means of allowing a choice between the two. In an effort to resolve this question, we subjected tissue samples of the 2 taxa to a battery of electrophoretic techniques (modified buffer systems, acrylamide gels and isoelectric focusing) with no success and we are unable at this time to distinguish between the 2 populations on the basis of allozymes. Fortunately, preliminary data from restriction endonuclease analyses of mitochondrial DNA of these species are available[13]. These data indicate that *C. b. stictogrammus* and *C. costatus* have quite different mtDNA and that *C. exsanguis* has the *C. costatus* mtDNA genome. Thus, it is reasonable to conclude that not only was *C. costatus* involved in the hybridization but that it was the maternal parent involved in the original hybridization with either *C. inornatus* or *C. septemvittatus*. The resulting diploid form is not known. Although it may never be determined, we suspect that *C. inornatus* was the species involved in the first hybridization (fig). There are other parthenogenetic populations that undoubtedly contain a *C. costatus* allozyme genome but have a *C. inornatus* mtDNA genome[13], which may represent reciprocal crosses to this one. *Cnemidophorus inornatus* and *C. costatus* do not now occur sympatrically[14], but they undoubtedly did in the not-too-distant past. It is known, for example, that the geographical range of *C. inornatus* has contracted considerably in just the last 100 years[15] and that, even in historical times, favorable habitat for *C. inornatus* was present in northeastern Sonora[16]. It may also be that the presence of 6 parthenogenetic species in the immediate area may have modified the distribution of these 2 bisexual species. Regardless, the resulting diploid parthenoform was present long enough to have hybridized with another species, *C. septemvittatus*. To have done this, it may have had an extensive geographical range.

In conclusion, the analysis of alleles at 19 polymorphic loci in *C. exsanguis* and 7 potential parental progenitors indicates that *C. exsanguis* is an allotriploid resulting from hybridization involving 3 species. These 3 species are *C. costatus* (♀), *C. inornatus* (♂) and *C. septemvittatus* (♂), respectively.

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Experiments on sexual isolation between Chilean and European strains of *Drosophila subobscura*

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Summary. Males and females of Chilean strains of *Drosophila subobscura* exhibit a pronounced tendency toward homogamic mating. This tendency shows a clear relation to the distance between the geographic localities from which the respective strains came. Nevertheless, when the Chilean flies are confronted with European strains, the ethological isolation is observed in some cases but not in others, depending on the geographic origin of the strains.
Key words. Drosophila subobscura; mating isolation; sexual isolation; behavior genetics.

Drosophila subobscura is a very recent colonizing species in Chile[2,3]. It was detected for the first time in 1978. Its distributional area now extends continuously from La Serena (Lat. 29° 54′ S), located in the semi-desert and temperate northern zone of Chile, to the extremely cold and windy conditions existing near Punta Arenas on the Strait of Magellan (Lat. 53° 40′ S). Recent experiments by the present authors have revealed rudiments of sexual isolation between different Chilean populations of *D. subobscura*. It is well known that mating behavior in the *Drosophila* genera are under the control of genetically determined factors, mainly polygenes, and it could be modified by selective pressures[4-6]. The objective of the present paper is to discuss the results of these studies, and to report the results of experiments on ethological isolation between Chilean and European strains of *D. subobscura*.

Materials and methods. We used 4 Chilean strains of *D. subobscura* which originated from a large number of flies collected about 1 year before the initiation of the study in the localities

Table 1. Matings observed in multiple choice experiments among different Chilean strains of *D. subobscura* and the Levene's isolation coefficients

Strain 1	Strain 2	Runs	Pairs mated	% mated	♂1×1♀	♂2×2♀	♂1×2♀	♂2×1♀	Z_I	Z_m	Z_f
Santiago	S. Del Laja	10	83	41.5	17	31	19	16	1.31 ± 0.26	0.80 ± 0.20	0.68 ± 0.18
Santiago	Pto. Montt	10	117	58.5	22	51	19	25	1.53 ± 0.24*	0.57 ± 0.14**	0.75 ± 0.17**
Santiago	Coihaique	10	123	61.5	46	41	15	21	2.44 ± 0.31**	0.89 ± 0.18	1.25 ± 0.22
S. Del Laja	Pto. Montt	10	122	61.0	43	33	28	18	1.67 ± 0.24**	1.42 ± 0.22**	0.91 ± 0.18
S. Del Laja	Coihaique	8	77	48.1	37	16	13	11	2.03 ± 0.36**	1.65 ± 0.32**	1.39 ± 0.29**
Pto. Montt	Coihaique	10	114	57.0	52	20	28	14	1.62 ± 0.26*	2.28 ± 0.31**	1.14 ± 0.41

Z_I, joint isolation index; Z_m, male selection index; Z_f, female selection index; $Z = 1$, random mating; $Z > 1$, homogamic tendency or advantage of strain 1; $Z < 1$, heterogamic tendency or advantage of strain 2; *$p = 0.05$–0.005; **$p < 0.005$.

Santiago (Lat. 33° 30′ S), Salto del Laja (Lat. 37° 10′ S), Puerto Montt (Lat. 41° 28′ S) and Coihaique (Lat. 45° 34′ S). In addition, we used 4 European strains also maintained in mass cultures for several months. Two of these strains, Tübingen (W. Germany) and Cinisi (Sicily), were provided by Prof. D. Sperlich from the University of Tübingen, the 2 others Mallorca and Almería (Spain), by Prof. A. Prevosti from the University of Barcelona and Prof. R. de Frutos from the University of Valencia, respectively.

The experiments were performed at controlled room temperature (23 °C), in the morning, by direct observation of the matings in chambers of the type devised by Elens and Wattiaux[7]. Ten pairs of 8-day-old virgin females and males of one strain, plus 10 pairs of virgin females and males of a 2nd strain – 20 pairs in all – were introduced without anesthesia into the chambers and the 4 possible types of matings (♂ 1 × 1 ♀, ♂ 2 × 2 ♀, ♂ 1 × 2 ♀ and ♂ 2 × 1 ♀) observed during the first 90 min after the initiation of the experiment were recorded. In order to distinguish between flies of the 2 strains, the thorax of males and females of one of the strains was labelled with a tiny spot of white plastic paint. The experiment was repeated 8 to 10 times for each combination of strains. In half of the experiment flies of the first strain were labelled and those from the second remained unlabelled; in the other half it was the reverse. The painted spots were found not to affect the mating propensities.

For analysis of the data, deviations from the random mating expectations were estimated by the χ^2-test. We also used Levene's coefficients of isolation and selection[8] to analyze jointly or separately the homogamic or heterogamic mating propensities of males and females from each strain. Complete descriptions of the statistics employed are given by Ehrman and Petit[8] and Petit et al.[9].

Results. Table 1 gives the isolation coefficients observed between Chilean populations of *D. subobscura* in the multiple choice experiments. Evidences of ethological isolation were observed between the strains and the tendency to homogamic mating was statistically significant (χ^2-test for 3 df) in all cases except one. This tendency seems to be a direct function of the geographic distance between populations. The Santiago and Coihaique strains, the geographically most distant ones, exhibit the highest joint isolation index whereas the lowest index is found for the Santiago and S. del Laja strains which are the geographically closer populations.

Table 2 shows that the Santiago strain is not significantly isolated from 3 out of 4 European populations (Tübingen, Almería and Mallorca) but a strong isolation from the Cinisi strain from Sicily is observed. The Coihaique strain on the other hand shows a significant sexual isolation from Almería and Mallorca strains but not with those from Tübingen and Cinisi. The European strains show no significant tendency for homogamic mating when confronted with each other.

In conclusion, the Chilean populations seem to be sexually more isolated from each other than from the European populations, whereas no indications for any ethological isolation were found within the European populations.

Regarding to the male (Z_m) and female (Z_F) selection coefficients, there appear to be more cases of statistically significant deviations from random mating for males than for females, among the Chilean populations as well as when Chilean strains and European populations are brought together. This seems to indicate that, in general, males are more discriminative than females.

It is important to remember that many of the observed deviations from random mating are significant because of the different mating propensities of males and of females of the different strains utilized. In general, Chilean flies are sexually less active than the European ones, with the exception of those from Tübingen. The most active flies seem to be those of Cinisi (Sicily) and the least active those from Santiago and from Tübingen. These latter flies show a non-significant joint isolation index with regard to the 3 strains with which they were confronted (2 Chilean and 1 European).

Discussion. Evidences of non-random mating between geographic strains of *Drosophila* have been observed in some species but not in others. For example, no evidence for ethological isolation has been found between distant populations of the

Table 2. Matings observed in multiple choice experiments between Chilean and European strains of *D. subobscura* and the Levene's isolation coefficients

Strain 1	Strain 2	Runs	Pairs mated	% mated	♂1×1♀	♂2×2♀	♂1×2♀	♂2×1♀	Z_I	Z_m	Z_f
Santiago	Tübingen	10	113	56.5	25	32	33	23	1.01 ± 0.18	1.05 ± 0.19	0.73 ± 0.16
Santiago	Cinisi	10	136	68.0	33	57	21	25	1.89 ± 0.25**	0.69 ± 0.15**	0.83 ± 0.16**
Santiago	Almeria	10	101	50.5	27	31	17	26	1.37 ± 0.23	0.75 ± 0.16	1.15 ± 0.21
Santiago	Mallorca	10	131	65.5	21	50	19	41	1.16 ± 0.20	0.44 ± 0.12**	0.95 ± 0.18
Coihaique	Tübingen	10	99	49.5	41	15	26	17	1.18 ± 0.23	2.04 ± 0.30**	1.33 ± 0.24*
Coihaique	Cinisi	10	150	75.0	33	51	28	38	1.25 ± 0.18	0.69 ± 0.13**	0.93 ± 0.16
Coihaique	Almeria	10	117	58.5	36	44	16	21	2.17 ± 0.29**	0.78 ± 0.17	0.96 ± 0.19
Coihaique	Mallorca	10	106	53.0	25	46	15	20	1.95 ± 0.29**	0.63 ± 0.16**	0.85 ± 0.19*
Cinisi	Tübingen	10	129	64.5	39	28	42	20	1.14 ± 0.19	1.71 ± 0.22**	0.81 ± 0.14
Almeria	Mallorca	10	114	57.0	34	30	27	23	1.28 ± 0.21	1.15 ± 0.20	0.98 ± 0.18

Z_I, joint isolation index; Z_m, male selection index; Z_f, female selection index; $Z = 1$, random mating; $Z > 1$, homogamic tendency or advantage of strain 1; $Z \leqslant 1$, heterogamic tendency or advantage of strain 2; *$p = 0.05$–0.005; **$p > 0.005$.

cosmopolitan species *D. melanogaster*[9,10]; or between geographic strains of the wide-ranging neotropical species *D. willistoni*[8,11]. On the contrary, in other species like *D. sturtevanti*[12], *D. pseudobscura*[13], *D. serrata*[14], *D. equinoxialis*[8] and the superspecies *D. paulistorum*[15] there exists a pronounced tendency toward homogamic mating. Further, in other species such as *D. prosaltans*[16] and *D. tropicalis*[8], the tendency of males and females to mate within the same strain shows a clear relation to the distance between the geographic localities from which the respective strains came.

With regard to mating isolation, *D. suboscura* seems to behave similarly to the last type of species. According to our results, different geographic strains of the species show significative homogamic tendencies in mating. This is especially clear when the 4 Chilean populations are considered. They show a North-South gradient of ethological isolation. In Europe, by a different method, Sperlich[17] demonstrated a North-South cline in mating preferences, which he related to the different levels of chromosomal polymorphism existing in the populations of *D. subobscura*. In general, in our experiments, the European strains show a lower tendency to homogamic mating, either when paired with each other or when combined with the Chilean populations. There is no good explanation for this. In other species, like the incipient species of the *D. paulistorum* complex[18,19] it was observed that the isolation between sympatric strains was almost without exception greater than between allopatric strains. A similar phenomenon might occur in *D. subobscura*. Although obviously all our *D. subobscura* strains are allopatric, it is important to bear in mind that the Chilean strains came from populations which are not yet geographically isolated from each other, but are, on the other hand, very far apart from their European ancestors.

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Strong mutagenic action of a bipyridylium herbicide in a N_2-fixing blue-green alga

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Summary. The herbicide paraquat (1,1′-dimethyl-4,4-bipyridylium ion) has been found to exert a growth inhibitory effect on the N_2-fixing blue-green alga *Nostoc muscorum* in nitrogen-free (N_2) and NO_3^- media, without any apparent inhibitory or stimulatory effect on the heterocyst-forming capacity of the organism. With a dose of paraquat permitting about 20 and 50% survival of this alga a reverse mutation (from $het^+ nif^-$ auxotrophy to $het^+ nif^+$ prototrophy), a forward mutation (for L-methionine-DL-sulfoximine [MSO]-resistance), and an auxotrophic mutation (for carbon-auxotrophy through methylamine [MA]-resistance) have been obtained. The toxic and mutagenic effects of this agrochemical have been compared with those of the well known mutagen MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) and found to be stronger than those of the latter in each case.

Key words. Paraquat; herbicide, bipyridylium; alga, blue-green; mutagenic action; *Nostoc muscorum*.

The current extensive interest in identifying potential environmental mutagens has resulted in intensive efforts to establish reliable screening tests for examining the possible mutagenic and/or carcinogenic actions of various synthetic agricultural chemicals and food additives[2–7] and a number of pesticides (including herbicides, fungicides, insecticides, molluscicides, raticides and nematocides) have been found to be mutagenic in bacterial, mammalian and higher plant systems[8–10]. However, effects on the naturally-occurring blue-green algae, which are invariably present at the time of herbicide application in the fields, and a majority of which are highly beneficial for rice agriculture in tropical countries owing to their significant N_2-fixing properties[11–13], have received little attention from biologists and mutageticists. The mutagenicity of only 2 herbicides, i.e. butachlor (2-chloro-2,6′-diethyl-N [butoxymethyl] acetanilide) and alachlor (2-chloro-2,6′-diethyl-N [methoxymethyl] acetanilide) has been established so far in a N_2-fixing blue-green alga *Nostoc muscorum*, by the author's group[14,15].

The bipyridylium ion is known to liberate free-radicals during its interactions with living organisms, which makes it possible that it may have potent genetic effects[16]. This prompted the author to examine whether a bipyridyl compound can cause genetic alteration to any extent in a blue-green algal system. Paraquat is one of the most popularly used bipyridyl herbi-