GENETICS OF ETHOLOGICAL ISOLATING MECHANISMS IN THE SPECIES COMPLEX JAERA ALBIFRONS (CRUSTACEA, ISOPODA).

M. Solignac

Lab. de Biologie et Génétique Évolutives, C.N.R.S.

Gif-sur-Yvette, France.

Introduction

The Jaera albifrons complex includes five related species found in cold and temperate coastal regions of the North Atlantic. The species are limited to the intertidal zone and live under stones or among algae.

Females of the five species are morphologically indistinguishable, while differences in the male pereiopods allow specific identification (Bocquet, 1953). In each species, the sexual variants (secondary sexual characters) are closely related to the stimulus-signals emitted by the male during the precopulation phase (Solignac, 1975).

Three species, Jaera (albifrons) albifrons, J. (a.) ischiosetosa and J. (a.) praehirsuta have a widespread distribution on both sides of the Atlantic. The two other species, J. (a.) posthirsuta and J. (a.) forsmani are limited to the east coast of North America and to western Europe respectively.

It is fairly easy to obtain interspecific hybrids in the laboratory when no choice of mate is allowed. The ten possible hybrid combinations and their reciprocals can all be obtained but with varying degrees of success. In nature, however, where mixed populations are not uncommon, the frequency of hybrids generally does not exceed 1%.

In the absence of temporal (Jones and Naylor, 1971),

mechanical and gametic isolation, the sexual barrier guarantees
specific integrity in mixed populations. Hybrids of the
first generation are usually perfectly viable, except in some
crosses (and then only in one direction) where females (the
heterogametic sex) are produced in reduced numbers. The
F_1 hybrids produce numerous and apparently normal gametes, but
offspring of hybrids crossed among themselves or with parents
are significantly fewer in number (Solignac, 1976).

The present work is limited to a study of sexual isola-
tion. Some aspects of the genetic determination and of the
variation between pairs of species of this isolating mechanism
are analysed. In the sexual behaviour of this isopod, two
steps are likely to be important (Solignac, 1975):

1. Discrimination by males during pair formation:
   males take the initiative and the study was made
   by the male choice technique.
2. Female reactivity to heterospecific overtures:
   studies were made on isolated heterospecific couples.

Sexual Choice by Males

The male choice technique has been widely used for the
study of the degree of isolation between species of <u>Drosophila</u>
and of the incipient isolation between geographic strains
(reviewed by Anderson and Ehrman, 1969). Results have gene-
rally been based upon the percentage of homogamic and hetero-
gamic insemination. In <u>Jaera</u> <u>albifrons</u>, ethological isolation
between species is very strong, both in nature and in the
laboratory. As observation of heterospecific impregnation in
<u>Jaera</u> would be relatively infrequent, this study was limited
to pair formation. This is, of course, only one component
of isolation, but the level of discrimination shown by males
is important in avoiding the formation of heterospecific cou-
ples with consequent wastage of time by partners in a pairing
without issue.

<u>Analysis of sexual choice</u>. Some preliminary experiments
were performed before the technique was standardized in order
to establish the optimal conditions for repeatability, and to
establish the limits of reliability of the data. Two popu-
lations belonging to different species, <u>ischiosetosa</u> and
<u>albifrons</u>, both originating from Kiel (Germany) were studied
in some detail.

Equal numbers of females of the two species (20 of each) were introduced into a sea water tank. Females, being morphologically indistinguishable, were identified by genetic markers. A choice was then given to a sample (generally about 40) of the males of the first species: the test was considered as positive when a male mounted a female in the "head-to-tail" position characteristic of the initial precopulation phase in the Jaera albifrons complex. The two partners were then separated, the male was removed and the female put back in the tank. The nature of the female chosen was noted. After 20', most of the males had made a choice and had been substituted by males of the second species which were tested in the same manner.

These experiments were repeated several times with the same animals, intervals of one week separating each test. Results were generally homogeneous and summed.

Following this protocol, a series of nine experiments was performed on these two populations using different generations, over a period of more than four years. The results are shown in Table 1. They are homogeneous for albifrons and slightly heterogeneous for ischiosetosa. Nevertheless, considering the diversity of tested animals, particularly in body size and in the genetic markers carried, the results may be taken as reasonably consistent. Another presentation is given in Figure 6: the variability observed for this pair of populations is small compared to those obtained by changing the nature of populations.

The data indicate two rather extreme cases: albifrons males appear to be indifferent in their choice of female, while ischiosetosa males choose their own females far more often than alien ones, showing only an average rate of error of twenty percent.

The nine experiments described above were performed with animals isolated from birth and replaced in individual dishes after each test. Other males extracted from mass cultures, and kept with their own females between tests were also studied. The ninth experiment in Table 1 was the control (isolated males) for this experiment. Males extracted from mass culture were less active than isolated males (Table 2). In both species, males from mass culture showed more pronounced homogamic tendencies than isolated males.

From these results, it can be concluded that the degree

TABLE 1

Choice by <u>ischiosetosa</u> and <u>albifrons</u> males between <u>ischiosetosa</u> (♀ I) and <u>albifrons</u> (♀ A) females and percentages of albifrons females chosen.   Each experiment represents the sum of several tests.

DATE	ISCHIOSETOSA MALES			ALBIFRONS MALES		
	♀I	♀A	%♀A	♀A	♀I	%♀A
IV 72	81	10	11	53	50	51
VIII 72	24	4	14	18	24	43
II 73	59	11	16	40	41	49
V 73	150	34	18	65	78	45
V 74	133	27	17	197	168	54
X 75	71	17	19	50	42	54
II 76	196	39	14	80	69	54
IX 76	216	63	23	392	267	59
X 76	414	137	25	390	314	55
MEAN			17.5			51.8
TOTAL	1344	342	20.3	1285	1053	55.0

Homogeneity

$$\chi^2_8 = 17.99 \qquad\qquad \chi^2_8 = 14.98$$

$$0.01 < P < 0.05 \qquad\qquad P > 0.05$$

TABLE 2

Sexual activity of and discrimination by <u>ischiosetosa</u> and
<u>albifrons</u> males. For both species, isolated and non-
isolated males were tested.

		% of males having chosen:		ischiosetosa females chosen	albifrons females chosen	% ♀ A
		20'	60'			
albifrons males	isolated	89		314	390	55.4
	from mass culture	42	63	202	304	60.8
ischiosetosa males	isolated	92		414	137	24.8
	from mass culture	18	32	170	21	11.0

of sexual choice of males, for a given pair of populations, is reproducible in defined conditions and is hereditarily transmissible.   Learning cannot be excluded, but the greater discrimination of males with previous contact with females is perhaps a secondary result of their lower sexual activity.

A modified technique was used in an attempt to search for variation in the sexual choice of males.   Results from experiments of this sort could reveal behavioural heterogeneity of males, some of them with homogamic tendencies, others being indifferent or preferentially heterogamic.

Males of the two species, isolated from birth, were allowed five successive choices at intervals of one week.   After five experiments, males were distributed in six classes based upon having chosen one kind of female (in this case ischiosetosa) 0 to 5 times.   In Figure 1, the experimental distributions are compared to the theoretical binomial ones calculated with the same respective mean of choice.   The experimental distributions for the two species, for the five successive tests are not significantly different from expected.

Although the technique is far from sensitive these results suggest the absence of any obvious intra-specific genetic variability for this behaviour.   All males seem to have the same probability of error in making their choices.

Using this experimental procedure, strictly homogamic or heterogamic albifrons males were selected.   After the four generations of selection so far completed no consistent results have been produced in promoting choice of their own or alien females.   The number of generations of selection is low, but these early results are not in conflict with the inference drawn from the shape of the distributions in Figure 1.

Genetic determination of sexual choice.   As the variation in sexual choice is weak in parents, the study of the genetic determinism of these choices must be approached by the shift of interspecific hybrids.   Two independent experiments were performed.

In the first experiment, pure albifrons and ischiosetosa males and F_1 hybrid males were given a choice between hybrid and/or pure females (Table 3).   The results were as follows:

ischiosetosa males always rejected albifrons females and apparently confused hybrid females with their own;.
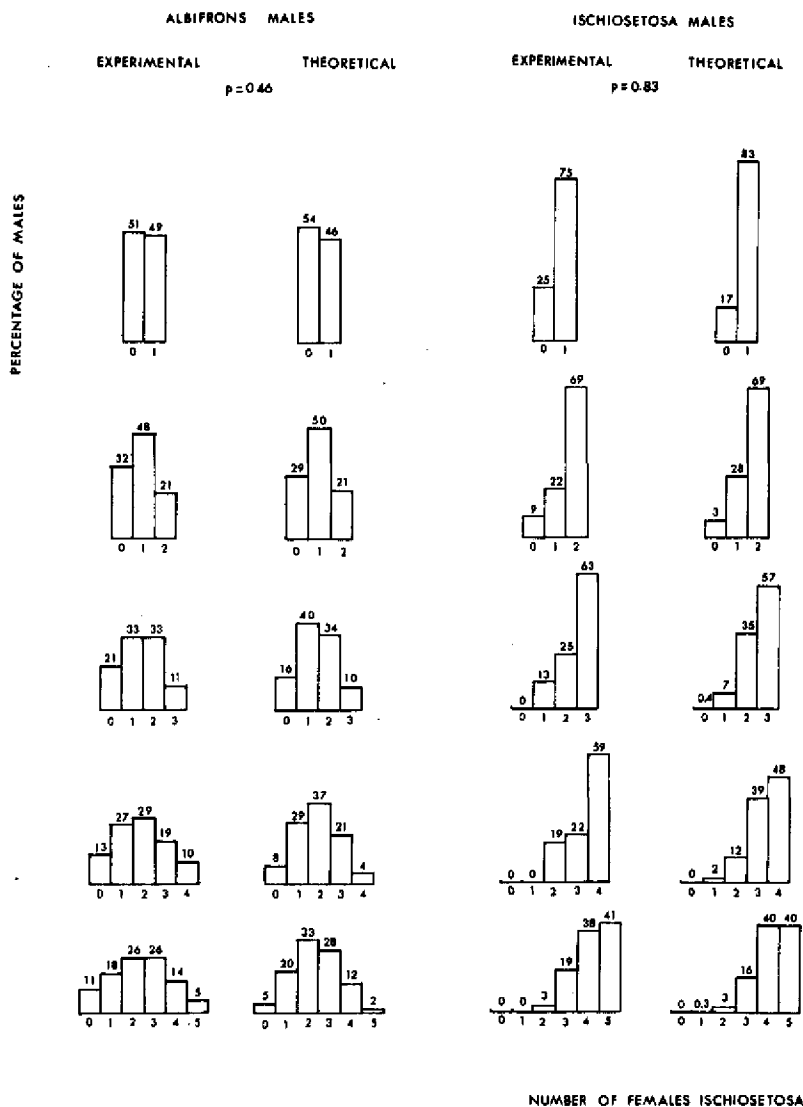
Figure 1. Experimental and theoretical binomial distribution in five successive mate choice experiments with <u>albifrons</u> and <u>ischiosetosa</u> males. The theoretical distribution is established using the value of <u>p</u> obtained as a mean of the five experiments.

TABLE 3

Choices by <u>ischiosetosa</u>, <u>albifrons</u> and F_1
hybrid males between hybrid and/or pure-
bred females.

♀ \ ♂	I		A		IA		AI	
	N	%	N	%	N	%	N	%
I	133	83	168	46	212	55	93	53
A	27	17	197	54	173	45	82	47
		*				*		
I	137	55	95	45	169	41	77	44
AI	114	45	118	55	244	59	98	56
						*		
AI+IA	78	89	74	45	167	54	81	58
A	10	11	89	55	144	46	59	42
		*						
Σ% /3 I		46		30		32		32
A		9		36		30		30
Hyb.		45		33		38		38

* P < 0.05

– <u>albifrons</u> males, as in the preceding experiments, showed a low discriminatory capacity.

– hybrid males obtained from the two reciprocal parental crosses showed the same behaviour with, generally, a slight preference for the hybrid females.

A second experiment was performed with pure males and various types of hybrid males, but with a choice only between parental female types.   18 kinds of males were tested:

– pure <u>albifrons</u> and <u>ischiosetosa</u> males;

– reciprocal hybrids of the first generation;

– second generation hybrids from crosses ♀ <u>AI</u> x ♂ <u>AI</u>;

— male products of the three first generations of re-
current or non-recurrent back crosses (BC).

The back cross males were obtained by crossing hybrid
females AI to the two types of pure males (BS1 $\underline{I}$ and BC1 $\underline{A}$);
female offspring of these two back crosses were then crossed
either to <u>ischiosetosa</u> or to <u>albifrons</u> males, giving four kinds
of BC2: $\underline{II}$, $\underline{IA}$, $\underline{AI}$ and $\underline{AA}$, considering only the nature of males
to which F1 and BC1 females were back crossed.   Of the 8 kinds
of the third back cross, only seven were tested, the number of
$\underline{IIA}$ males being too small to test.

Males can be characterized by an <u>average</u> genetic constitu-
tion, in terms of the percentage of genes of one species they
carry (in this case <u>albifrons</u>).

The correlation between the percentage of <u>albifrons</u> fe-
males chosen and the genetic constitution of the males was
very strong ($r = +0.92$) (Fig.2).   The experiment involved a
total of 4,376 choices.

It can therefore be concluded that there is genetic de-
termination of male sexual choice.   A monofactorial deter-
mination cannot be totally excluded because each point cor-
responds to a mean based upon numerous males (45 as an average
for each category).   It seems, however, reasonable to attri-
bute a polyfactorial basis to this character.

<u>Variability in sexual choice of males</u>.   Male choice
experiments were carried out with a number of populations
belonging to the same species or to different species.
Genetic markers were again used to recognise the two kinds of
females.   Intrapopulational references were also needed to
test the effects of these markers.

Choice indices of males 1 and 2 were calculated.   These
two categories were males of different colour morphs within
the same population or were individuals from different popu-
lations within the same species or were individuals of dif-
ferent species.   Male 1 choices were classified as homogamic
(or positively assortative) if females of type 1 were chosen,
heterogamic (or negatively assortative) if females of type 2
were chosen.   The choice index is the difference between the
number of homogamic and heterogamic choices, divided by the
sum of the two.   The index ranges from -1 to $+1$, positive
values denoting homogamy, negative values heterogamy, and a
zero value, random choice.   This simple index is derived
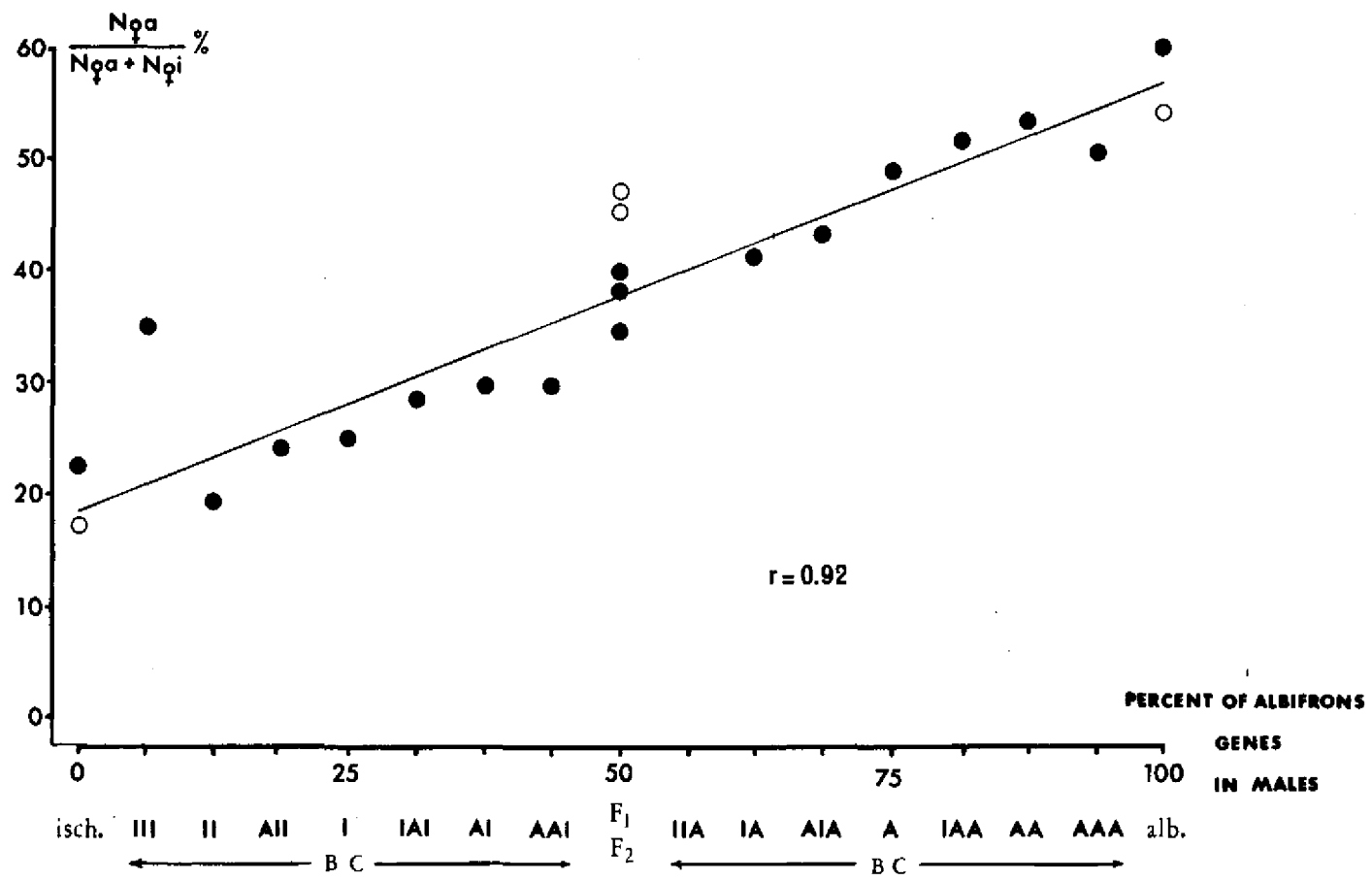from the isolation index of Stalker (1942) calculated on the

Figure 2. Choice between _ischiosetosa_ and _albifrons_ females shown by pure-bred males, F₁ and F₂ hybrid males and males produced by the three first back crosses. Open circles represent results obtained with the experiments reported in Table 3.

percentage of inseminated females.   The reciprocal index
is calculated in the same way for males of type 2.

The results are presented graphically (Fig.3): the index
of male 1 is plotted along the abscissa, male 2 being plotted
on the ordinate.   The two kinds of males are permutable and
there is a symmetry along the NE-SW axis.   The male with the
highest index was always designated as male 2 (all points are
on the same side of the axis of symmetry).   The joint choice
index (half of the sum of the two reciprocal indices) can be
read on the NW-SE diagonals.

The distance of points from the axis of symmetry gives
the attractivity index of females (half of the difference
between the two reciprocal indices, derived from Bateman's
index, 1948).   According to our convention, the index is
positive for female 2, negative with the same absolute value
for female 1.   Absolute values are on the same scale as the
joint isolation index.

The positions of points on the plan define immediately
the quality of the choice.

(a)   Intrapopulational choice: the two sexes were marked by
genes of the natural polychromatism of these isopods.
Variations affect the colour of the pigment, the pattern of
chromatic cells distribution, the development of chromogranules
and the degeneration of the pigmentry system (Bocquet, 1953;
Cléret, 1970).   Alleles controlling the phenotypes were main-
tained, not in separate stocks, but in a polymorphic state
in mass culture.   The so-called wild type phenotype (Cléret,
1970) nigrum granulatum uniforme is characterized by black
chromatophores uniformly distributed.

The deviations are relatively moderate (Fig.4) compared
with random choice: most of the points occupy a very small
area, near the origins.   Nevertheless, the nonrandomness is
statistically significant (P = <0.05) in 7 out of 28 chi-
squares (calculated for males 1 and 2 in 14 experiments):
in some instances selective choice exists.   One kind of
male (male 2 according to our convention) chose the category
of female marked by the same allele as he possessed (female 2)
(homogamy).   The second kind of male also preferred the type
2 females (heterogamy).   The choice shown by males was in-
dependent of the genes they carried, but one kind of female
was more attractive to the two kinds of males.

In the genus Drosophila, experiments using mutant and
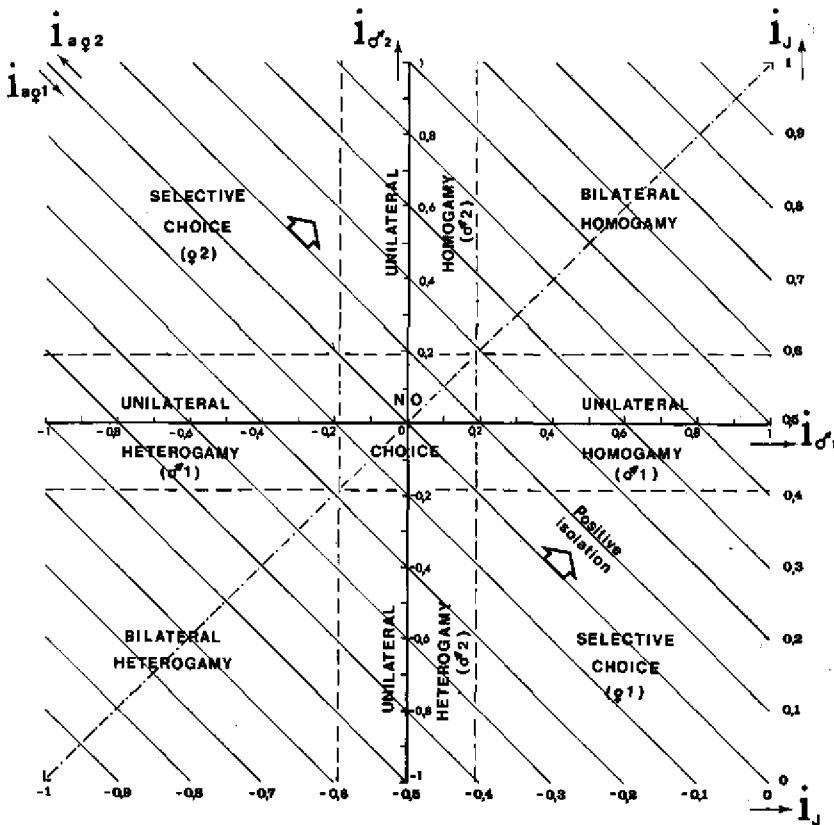wild-type stocks have demonstrated discrimination by females,

Figure 3. Graphic representation of male choice. Reciprocal indices of choice (i δ_1 and i δ_2) are plotted along the coordinates. Joint isolation index and female attractivity can be read along diagonals. Broken lines indicate limits of significance of χ^2, compared with random choice, for an arbitrary number of 100 choices. The occurence of points in various sectors indicates the quality of choices.
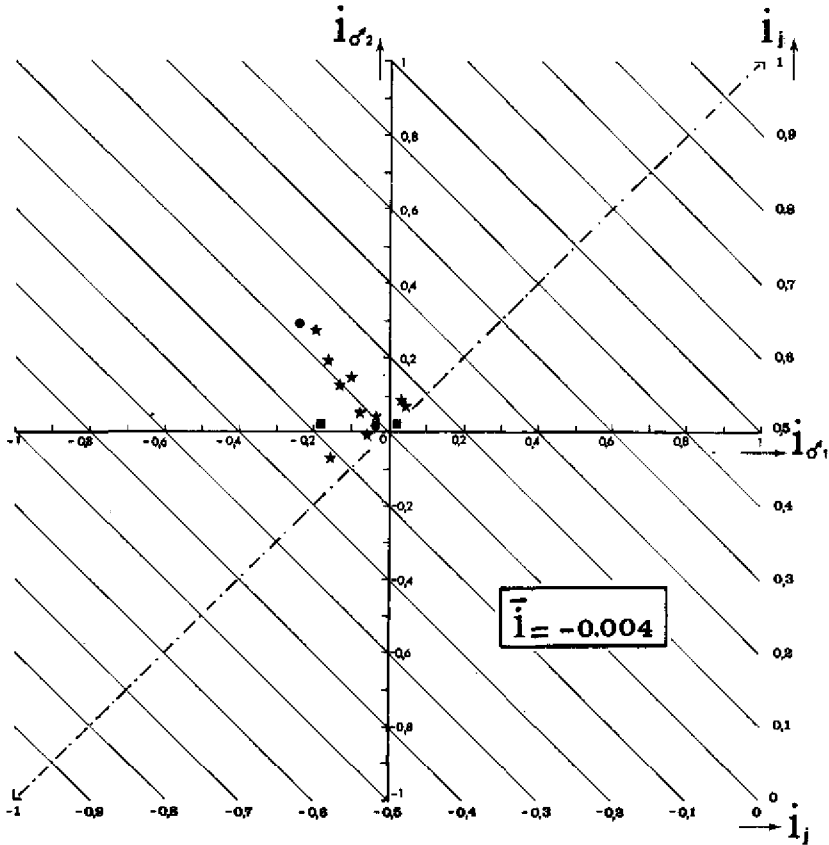
INTRAPOPULATION

Figure 4. Distribution of points observed with individuals
marked by genes for colour polymorphism within populations
(circles: albifrons, stars: ischiosetosa, squares: praehirsuta).

and the absence of male discriminating ability (Bateman, 1948).
In his review, Spiess (1970) concluded: "genotypes of males
may be selectively different and females are the selective
agents in the process".   The conclusions in Jaera are dif-
ferent but the technique used does not show whether any dis-
similarity exists in the courtship propensity of males.

Some other points of interest, still under investigation,
appeared during this experiment.

The most notable is that selective choices (combining both
significant or non-significant deviations) are in favour of
wild type females in 9 cases out of 10 (four other experiments
were performed with non-wild type animals).   In the last one
(praehirsuta of Woods Hole), Aurantiacum females were preferred.
It is interesting to note that this phenotype has a high fre-
quency in this strain.

Genes affecting the colour polymorphism of Jaera have
pleiotropic effects on behaviour.   Colour alleles do not
appear to influence the extent of isolation (the average index
of choice is -0.004) but may, in some cases, influence selec-
tive choice.

(b)   Interpopulation choices: the results of these tests are
illustrated in Figure 5, and are seen to be very different
from the intrapopulation tests.   Some pairs of populations
show an absence of choice in a few cases, but frequently one
type of male and often the two types show homogamic tendencies.
Considering couple formation, the existence of an incipient
isolation between geographic strains within a species can be
concluded.   The average index is + 0.200.   Further analysis
of data indicates a lack of correlation between the geographical
distance separating populations and the degree of isolation.

(c)   Interspecific choices: the average value is just a little
higher (+ 0.279) and the distribution of points in Figure 6
is very similar to that of Figure 5.   For some pairs of popu-
lations, isolation is rather high: the number of heterospeci-
fic couples formed is reduced by the discriminatory faculties
of males.   In nature, isolation by male choice is probably
higher since previous contacts render males more homogamic as
we have seen.

Comparison of the three diagrams (Fig. 4, 5 and 6) allows
further conclusions.   Single genes influencing colour change
the attractivity of females, but do not generate isolation
within populations.   Incipient isolation between strains and
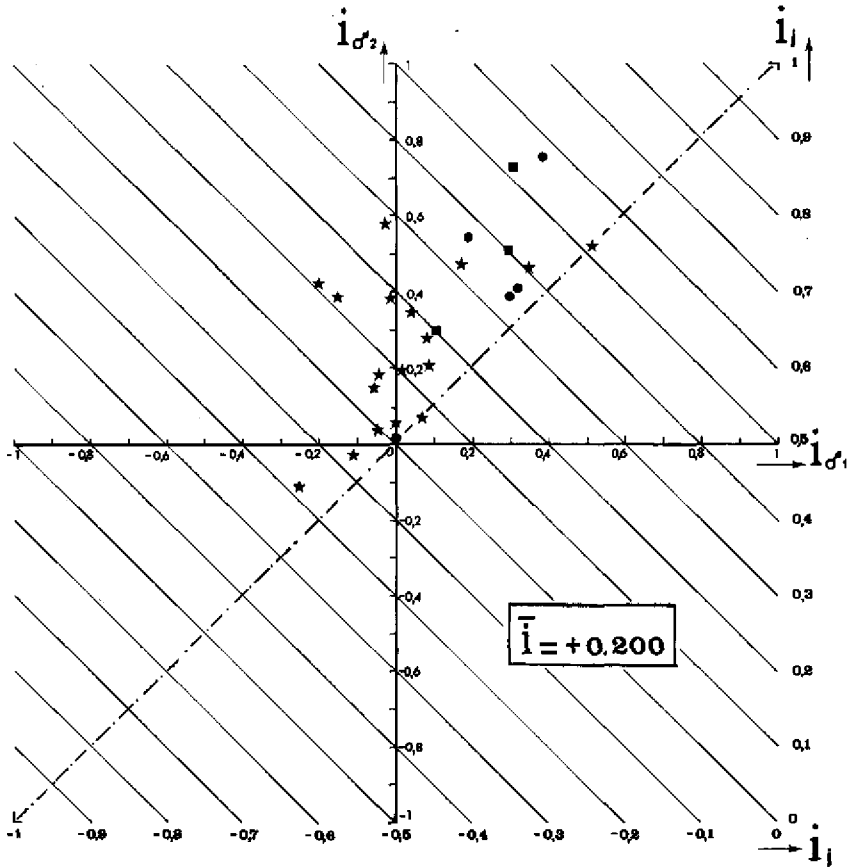
INTERPOPULATION

Figure 5. Distribution of points for mate choice between populations within species (same symbols as Fig.4).
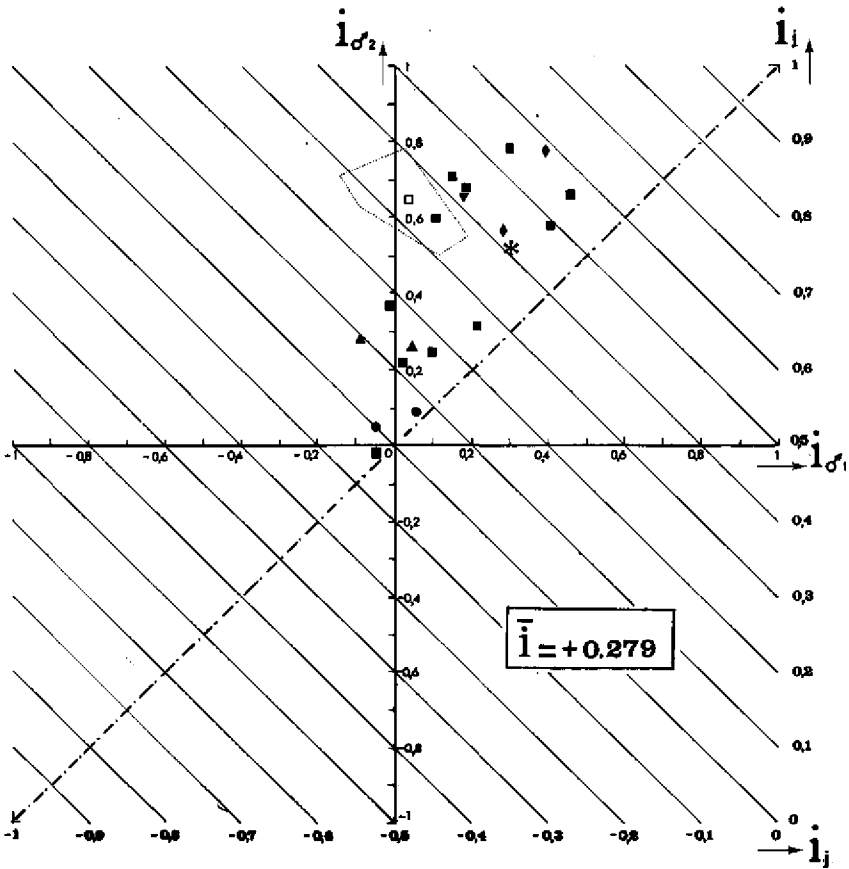
INTERSPECIFIC

Figure 6.   Distribution of points for mate choice between
different species.   Each symbol corresponds to a given pair
of species.   The dotted polygon is the envelop of points
obtained from data of Table 1 for ischiosetosa and albifrons
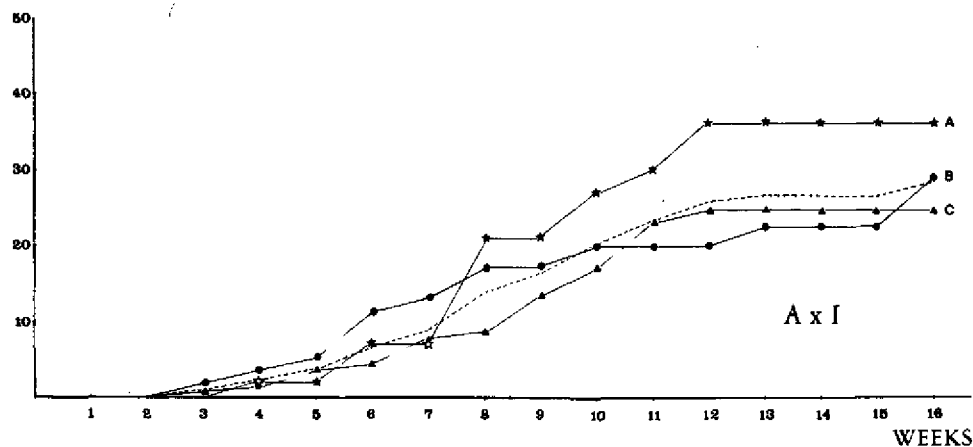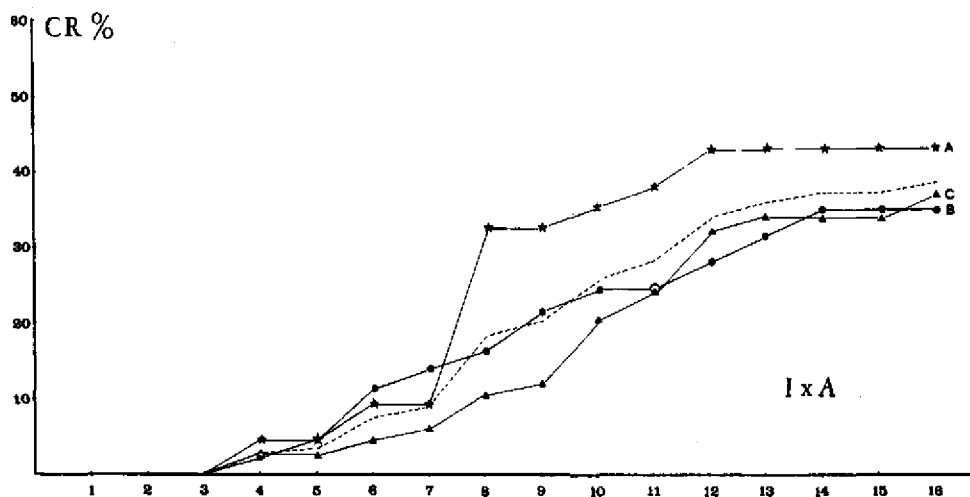populations from Kiel.

Figure 7. Crossing rate (CR) expressed as a cumulative percentage as a function of time (in weeks) in reciprocal crosses between <u>ischiosetosa</u> and <u>albifrons</u> (the first letter indicates the species of the female).

species (considering male choice) cannot result from the
simple accumulation of such point mutations.

The homogamy observable in interspecific choices is close
to that obtained between strains of the same species. These
incipient isolations must be considered more as a by-product
of the geographical divergence of populations rather than as
a consequence of a previous differentiation preceding or ac-
companying speciation.

Crosses without Choice and Female Reactivity

Using the same two populations of albifrons and ischiose-
tosa from Kiel, hybridizations were performed with hetero-
specific isolated couples. The success of crosses was esti-
mated by the appearance of hybrids. If couples are infertile
it is judged that impregnation has not taken place. Offspring
appear 2 to 5 weeks after insemination, depending on the time
of copulation in the female sexual cycle.

Analysis and repetitivity. The curves of Figure 7
depict the cumulative percentage of fertile couples over time.
This kind of presentation is preferred because once a female
has been inseminated, even by a heterospecific male, she con-
tinues to produce broods. These data were corrected to take
into account mortality (Solignac, 1976).

Hybridization progressed only slowly during the course
of weeks and even months. Three independent experiments were
performed, in each direction of crossing, with the same result.
The two reciprocal crosses give approximately the same results,
but this may not always be the case (see below). Reciprocal
hybridization rates can be considered as characteristic of a
given pair of populations.

The probability of finding new fertile couples among those
which were previously unmated seems to be more or less constant
over the weeks: the curve is damped as the number of virgin
females diminishes. This suggests the involvement of a random
process in the partial success of hybridization, rather than
a high receptivity of certain females to heterospecific sexual
overtures. This was also supported by complementary experi-
ments. Albifrons females were crossed with wild-type ischi-
osetosa males. As soon as a female produced a brood, the
male was changed for another ischiosetosa male bearing a domi-
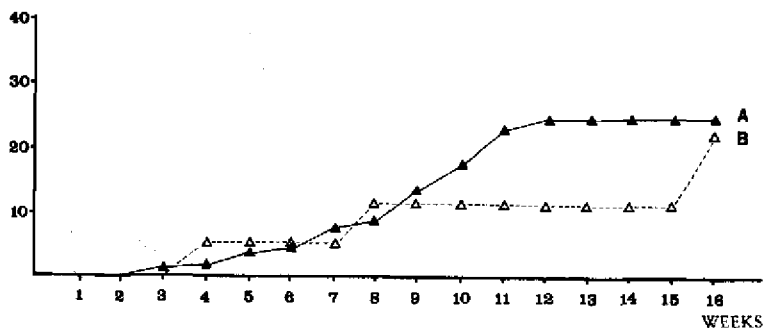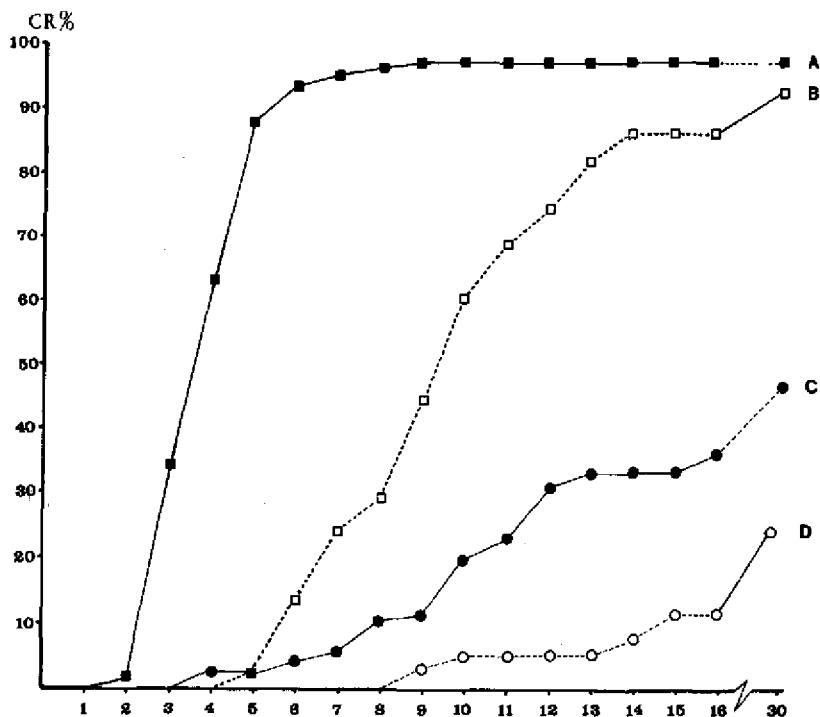nant marker. First and second heterospecific fertilizations

Figure 8. Crossing rate (cumulative percentage) as a function of time (in weeks) in various kinds of intra- and interspecific crosses: upper curves: ischiosetosa females: A: virgin ischiosetosa females crossed to their own males   B: already inseminated ischiosetosa females crossed to their own males (second conspecific insemination)   C: virgin ischiosetosa females mated to albifrons males: lower curves: albifrons females   A: hybridization of virgin albifrons females with ischiosetosa males   B: second interspecific insemination of albifrons females by ischiosetosa males.
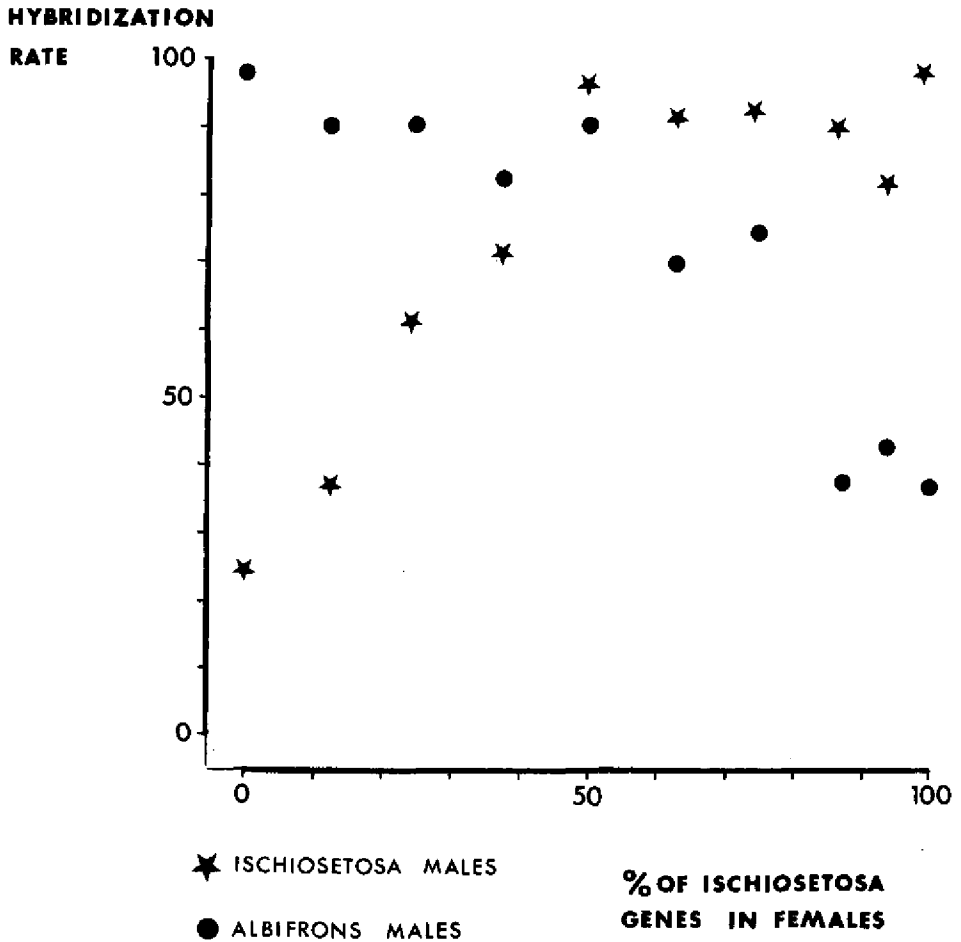
HYBRIDIZATION
RATE

Figure 9. Hybridization rate of <u>ischiosetosa</u> and <u>albifrons</u>
males with pure or hybrid females.   Hybridization rate
(percentage of successful crosses after 16 weeks, corrected
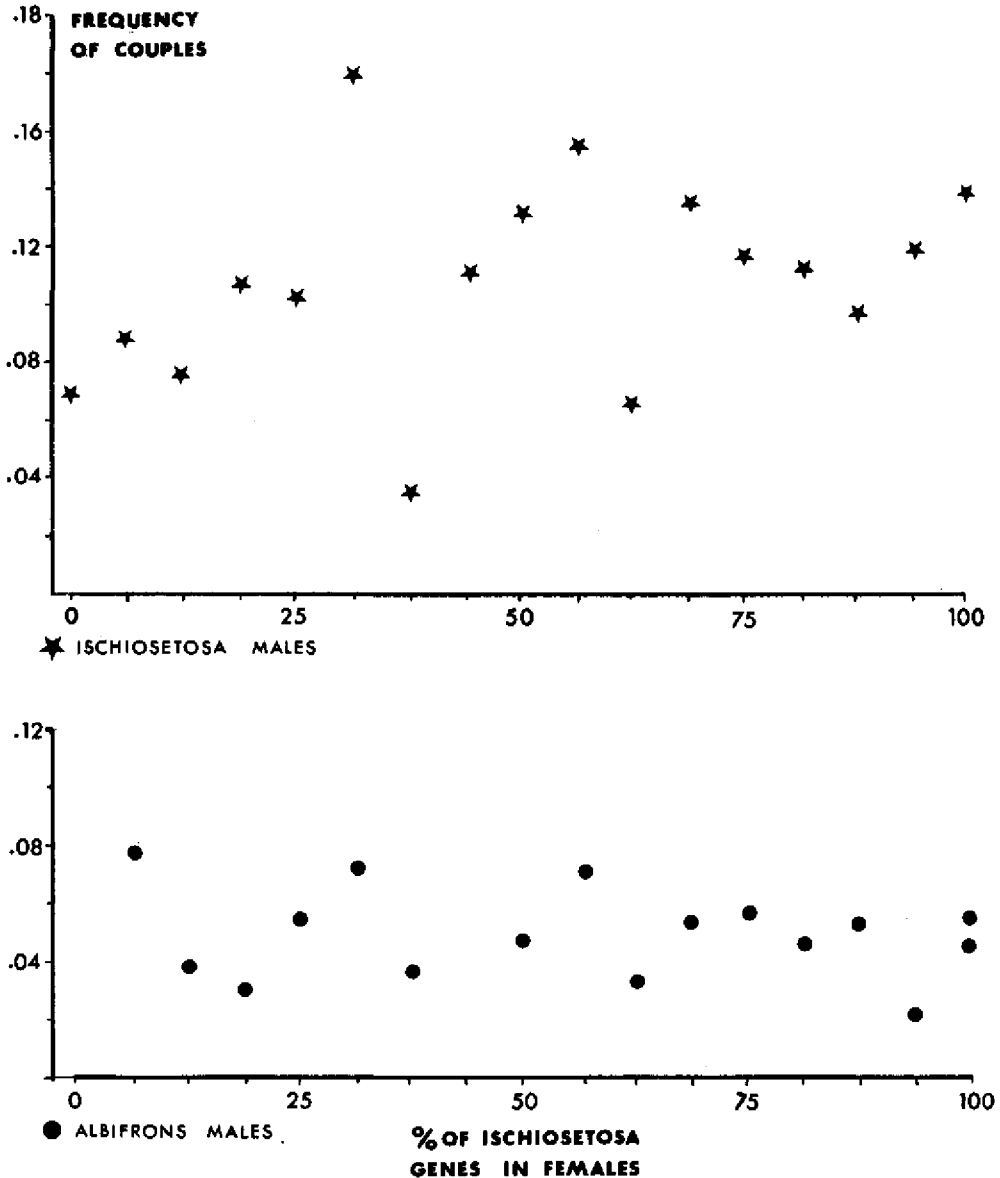for mortality) is expressed as a function of female genotype.

Figure 10.   Frequency of precopula phase observed during weekly examination of crossings for both _ischiosetosa_ and _albifrons_ males as a function of the genotype of the females to which they were crossed.

kinds of pure females.   In this case, the result of crossing
is evidently due to female reactivity to male stimuli.

With ischiosetosa males, the distribution of points is
not so clear, but a similar conclusion can reasonably be
drawn.

This kind of comparison must be limited to one type of
male with different types of females.   Indeed, the length of
precopulation is very variable between populations or from
one species to another.

It seems logical to suppose that the success of these
crosses, in the absence of choice, depends largely on female
reactivity.   Such a reaction is genetically determined but
it is not possible to conclude much as to the nature of the
genetical determinants.   Indeed, most of the results can be
explained by assuming the existence of two alleles, each of
them characterizing one species.   Nevertheless, as in the
male choice experiments, the hypothesis of a polyfactorial
basis of female reactivity is highly probable.

Variations of hybridization rate.   When varying the
pairs of species or even the populations within the species,
considerable variation in results was obtained.   Often, the
final hybridization rate was between 30 and 50%.   But female
posthirsuta originating from Newport (United States), crossed
with albifrons or ischiosetosa males as rapidly as they did
with their own.   On the contrary, praehirsuta males, from
Roscoff (France) produce a hybridization rate always close to
zero with ischiosetosa and albifrons females.

Some curves are given in Figure 11.   It can be seen that
the results obtained from the two reciprocal crosses are some-
times strikingly different.   For the moment, no general rule
can be drawn: the data are few due to the long time needed
for such experiments.

Conclusions

An attempt will be made to correlate the results obtained
by the two types of experiments.   When choice of mate is
available, the role of the two sexes is rather clear:

- due to male discrimination in certain pairs of
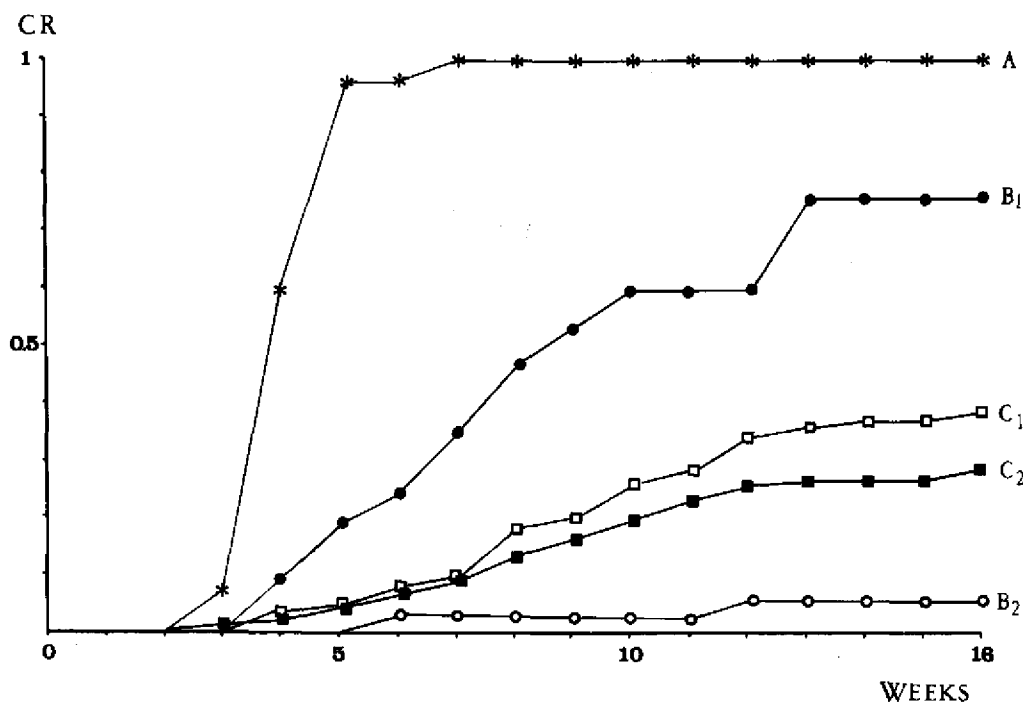populations, most couples are of the homogamic type;

<u>Figure 11.</u>   Crossing rate (CR, cumulative percentage) in various pairs of species: A: ♀ <u>posthirsuta</u> (Newport) x ♂ <u>ischiosetosa</u> (Ringkøbing). B_1: ♀ <u>praehirsuta</u> (Roscoff) x ♂ <u>ischiosetosa</u> (Kiel); B_2: reciprocal cross. C_1: ♀ <u>ischiose-tosa</u> (Kiel) x ♂ <u>albifrons</u> (Kiel); C : reciprocal cross.

- females are rapidly receptive to males of their own
kind and show a low receptivity to alien males;

- fertilized females are less receptive than virgin ones,
especially to heterospecific courtship.

When no mate - choice is allowed, the situation is not
so clear and three kinds of rather conflicting conclusions
can be drawn:

- in many experiments on hybridization, the rate of
fertilization depends on the discriminating faculties
of the male. The more discriminating the male during
choice, the lower the success of hybridization with
that male. Consequently, the role of the male seems
important.

- nevertheless, the frequency of precopula in isolated
heterospecific couples does not confirm the above con-
clusion. The attempts of males to copulate seem to be
characteristic of the kind of male and independent of
the genotype of the female. The reactivity of the
female seems most important in crosses, depending on
the female genotype.

- this last impression is also supported by inter-popu-
lation crosses. Males are sometimes very discrimina-
ting in their choices, as much as in interspecific
choices. Nevertheless, in the absence of choice they
fertilize females from alien populations very rapidly.

Consequently, the hybridization rate seems to be the con-
sequence of both frequency of attempts made by the male (male
activity) and of female reactivity. Nevertheless, in the
absence of choice, the female receptivity is probably the most
important factor.

More difficult, but also more important is the problem
in nature. The frequency of hybrids can be established in
males without difficulty, F1 hybrids and often products of
first back cross being morphologically identifiable. The
hybrid rate is generally between 1% and 0.1%. The actual
rate of hybridization in nature can also be established.
Hybrids in Jaera albifrons develop quite well under labo-
ratory conditions, as well in fact as the parental forms.
Offspring raised in the laboratory from inseminated females
collected in the field permit the estimation of the actual
hybridization rate. The frequency of hybrids in the off-

spring of such females is never higher than the hybrid fre-
quency observed in natural conditions among samples of males
collected at the same time as the females.   In other words,
the real frequency of hybridization is close to the observed
frequency of hybrids.

In the laboratory, in experimentally mixed populations,
species remain isolated with the same intensity as in nature
(Solignac, 1976).   Experimental analysis seems therefore to
give an accurate picture of what occurs in nature.   This is,
however, not a general conclusion valid for all organisms.
For instance, Drosophila pseudoobscura and D. persimilis, are
more isolated in the field than in the laboratory for reasons
which are not clear (see for example Dobzhansky, 1951).

                              Summary

     The complex Jaera albifrons includes five related species
of the cold and temperate coasts of the North Atlantic.   In
the laboratory, species can be hybridized if there is no
choice of mates, but in nature, they remain reproductively
isolated in the zones of contact or overlap.   A genetic study
of ethological isolating mechanisms has been carried out.
In Jaera albifrons, couples are established following male
initiative.   In male choice experiments, using males and fe-
males from the same population marked by different alleles of
loci controlling the natural colour polymorphisms, sexual
choice is either at random or is strictly selective i.e. one
kind of female is preferred by both types of males.   On
the contrary, interspecific choice, and at a lower level inter-
population preferences within a species, are of the homogamic
type.   The choice made by parental males and various kinds
of hybrid males (F1, F2 products of recurrent or non-recurrent
back crosses) show a genetic determination of sexual preferen-
ces, probably of a multifactorial nature.

     Female refusal to alien male stimuli is the main bar to
hybridization.   In the absence of mating choice, the success
of crosses between females or various degrees of hybrity and
parental males depends on the degree of genetic resemblance
between the two parents.   Full success of the cross is assured
if the female possesses at least 50% of genes in common with
the male.   Both under natural conditions and in laboratory
populations, the frequency of hybrids does not exceed 1%.
The frequency of hybrids, estimated from males, is a good in-
dex of the frequency of hybridization and hence the ethological
differences appear to be established as the main isolating
mechanism in nature.

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