Partial hybrid sterility between strains of the arrhenotokous spider mite, *Tetranychus urticae* complex (Acari, Tetranychidae)

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Abstract

Crosses between strains belonging to the *Tetranychus urticae* complex often reveal some degree of mortality in the eggs laid by the F_4 females. Two such strains were studied. Non-Mendelian features characterize the F_1 infertility: F_1 females of the same cross differ considerably regarding mortality in their eggs, reciprocal crosses give different results and the degree of egg mortality changes when the F_1 female gets older. Moderate changes in physical circumstances and the quality of the host plant have little or no influence on egg mortality. Haploid eggs, laid by virgin F_1 females, suffer considerably more mortality than eggs fertilized by sperm from the paternal or the maternal strain. Nine generations of repeated backcrossing to males of the original paternal strain did not result in a notable reduction of egg mortality, although evidence from another study (De Boer, unpublished) makes it highly unlikely that agents with a strictly maternal inheritance are involved.

Introduction

The phytophagous mites commonly referred to as the two-spotted spider mite, which form pests in orchards, gardens, glasshouses and on ornamental plants all over the world, are also highly problematic organisms for the systematic biologist. Pritchard and Baker (1955) in their revision of the spider-mite family Tetranychidae propose the name *Tetranychus telarius* (syn. *T. urticae*) for all these mites, but at the same time point out that it is 'a polytypic species represented by at least several subspecies or species'. Crossing experiments have been employed in order to clarify the systematic relations between the various forms, but these only revealed more complexity than was surmised before. Evidence that some degree of hybrid infertility occurs between virtually any two strains has been accumulating (Boudreaux, 1963; Helle & Pieterse, 1965; Overmeer & Van Zon, 1976; De Boer, 1980; De Boer, 1981). This hybrid infertility is expressed as a reduced hatchability of the eggs produced by the F_1 females, often accompanied by a reduced egg production.

Relatively much attention has been given to the occurrence of red and green forms. The name *T. cinnabarinus* has often been used for the red type and *T. urticae* for the green one. It seems that the F_1 of a red and a green strain is in general more sterile than the F_1 of two strains of the same colour (Van de Bund & Helle, 1960; Helle & Van de Bund, 1962; and Dosse & Nuber, 1963), but exceptions are known (Dupont, 1979; present study).

The present study concerns the characteristics of the hybrid infertility between two strains, a red one and a green one. It is an attempt to elucidate the underlying mechanism. A comparison is made with a seemingly related phenomenon in *Drosophila melanogaster*.

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Material and method

Strains and rearing method

The red and green strain will be referred to as the R and G strain respectively. The R strain has been in the laboratory for about twelve years and was originally collected near Ferrara, Italy (44° 50′ N 11° 38′ E). The G strain has been in the laboratory for about one year and was originally collected near Castricum, the Netherlands (52° 33′ N 4° 40′ E). In the laboratory the mites are reared on leaf cultures consisting of a detached leaf of bean *Phaseolus vulgaris* L. (Papilionaceae), pressed on a wad of cotton-wool soaked in a nutrient solution (Helle, 1962). The crossings were also performed on such leaf cultures. Unless stated differently the experiments were done in the same climatic room at a temperature of 27 °C and relative humidity of 70 to 80%.

Haplodiploidy

Spider mites exhibit haploid parthenogenesis or arrhenotoky, which means that males develop from unfertilized eggs and are haploid, having only a maternal set of chromosomes. The females develop from fertilized eggs; they are diploid with one paternal and one maternal set of chromosomes. Samples consisting of exclusively haploid eggs can be obtained by isolating unmated females on a leaf culture. Mated females produce haploid and diploid eggs in varying proportions.

Observations on individual F_1-females

Virgin females are obtained by isolating them in the last quiescent stage before adulthood, the *teleiochrysalis* stage. Crosses are performed by isolating one virgin female with one male on a leaf culture. Mating will soon take place. F_1 females are collected when they have reached the teleiochrysalis stage, eight days after egg deposition. A single virgin F_1 female is isolated on a leaf culture to deposit eggs during a four days' period. The eggs are counted immediately afterwards and viable progeny is scored during the subsequent week.

Mass crossing

The majority of the crosses were performed as mass matings, by isolating 15 virgin females in their third day of adult life, i.e. three days after eclosion from the teleiochrysalis stage, with an equal number of adult males. The next day ten of the inseminated females were transferred to a new leaf culture, where they were allowed to oviposit for on average 20 hours. After another seven days up to 30 pharate F_1 females were collected. They were left unmated or allowed to copulate with males of either the R or the G strain. In order to assess the hatchability of the eggs produced by these F_1 females ten of them, two samples from each mass crossing, were allowed to oviposit during on average 20 hours on a clean leaf culture. About 100 eggs are produced during that time. The eggs were counted immediately afterwards and the number of viable progeny was scored six days later. In case the F_1 females were inseminated the proportions of male and female progeny were established one day later. If F_1 females are kept alive for longer periods they have to be transferred to a new leaf culture once every week.

Statistical evaluations

Most of the egg mortality percentages concern the eggs produced collectively by ten females and can therefore be considered the weighted average of ten observations. In spite of this, the values obtained from replicate observations conform badly to a normal distribution. Therefore non-parametric statistical methods are always preferred. A non-parametric alternative was not always available for analyses of variance. However, as this technique is known to be a robust one, it is believed that useful information can be obtained by it. The names referring to the various non-parametric techniques are in accordance with Siegel (1956).

Results

Single F_1-females

Fifteen F_1 females from the crossing G♀ × R♂ were studied individually. From each one two samples of eggs were collected. The first sample consisted of the eggs laid during the fourth, fifth and sixth day of adult life. The second sample was collected later, on the tenth, eleventh, twelfth and

Table 1. Mortality in two egg samples from each of 15 virgin F_1 (G♀ × R♂) females from crossings between the G and R strains of the *Tetranychus urticae* complex. The 15 over-all mortality percentages are not homogeneous ($\chi^2 = 118$, d.f. = 14, $p \cong 0$). Mortality percentages of the first and the second period are often significantly different (χ^2 test, n.s.: not significant, *: $0.01 < p < 0.05$, ***: $p < 0.0001$). In the second period egg mortality is usually lower (Wilcoxon matched-pairs signed-ranks test, $p = 0.0006$, two-sided).

	Period 1		Period 2		
Female	3rd, 4th, 5th and 6th day of adult life		10th, 11th, 12th and 13th day of adult life		
	eggs produced	% not hatched	eggs produced	% not hatched	
i	55	67	19	11	***
ii	66	50	46	28	*
iii	74	15	64	6	n.s.
iv	74	38	39	44	n.s.
v	65	35	52	21	n.s.
vi	61	23	52	6	*
vii	63	57	51	12	***
viii	46	59	54	13	***
ix	72	60	12	17	*
x	56	21	60	18	n.s.
xi	74	46	38	8	***
xii	49	37	39	44	n.s.
xiii	30	47	25	32	n.s.
xiv	24	75	16	31	*
xv	63	59	18	33	n.s.

thirteenth day of adult life. Each one of the 15 females studied lived at a different time. This was so arranged in order to avoid systematic differences in treatment between on the one hand the first samples and on the other hand the second samples of eggs. The number of eggs in each sample and the mortality percentages are given in Table 1. Three features should be noted:

(1) The 15 F_1 females are not uniform, regarding the degree of inviability in their eggs. If the eggs of the first and the second period are pooled the proportions of inviable eggs of the fifteen females differ significantly ($\chi^2 = 118$, d.f. = 14, $p \cong 0$).

(2) Samples of the eggs, produced by the same female but during a different period of her life, are often significantly different with respect to the hatchability percentages.

(3) In most of the 15 cases the percentage of unhatched eggs in the second period is lower than it is in the first period. This is highly significant by Wilcoxon's matched-pairs signed-ranks test ($p = 0.0006$, two-sided). These features strongly indicate the involvement of non-Mendelian factors. The differences between various observations can possibly be ascribed to external circumstances, which modify the egg-mortality rate. This is, however, very unlikely in view of the results reported hereafter. In any case the general decrease in mortality in the two subsequent batches of eggs cannot be explained in any other way than by presuming that an internal change takes place in the ageing females.

Mass crosses

All the following data concern egg mortality percentages determined for the eggs collectively produced by ten females obtained from a mass crossing. Every observation is repeated several times. As expected the variation between these replicates is smaller than the variation between egg-mortality values of individual females. Nevertheless homogeneity tests reveal highly significant differences between replications of the same observation. Therefore it is not justified to draw conclusions based on the difference between two observations, even if this difference is significant. Only if repeated observations of one kind give systematically higher or lower values than a number of observations of another kind has a meaningful difference between the two kinds of observations been demonstrated.

Effect of ageing

The observations on individual F_1 females from the crossing G♀ × R♂ showed that ageing of these females affects their fertility i.e. the hatchability of their eggs. In order to obtain more detailed information egg samples from F_1 (G♀ × R♂)-females were taken at two, three, five, six, nine, twelve and fifteen days after the last ecdysis. As can be seen in Figure 1A, egg mortality becomes lower as the females get older. This correlation is significant by Spearman's rank correlation test ($n = 7, 0.01 < p < 0.001$, two-sided, considering the mean mortality in every age group). It is possible that relatively fertile F_1 females tend to live longer than the more sterile ones. In the present experimental set up this would also have the effect that more fertile eggs are obtained from older females. However, the good quantitative agreement with the observations on individual females suggests that

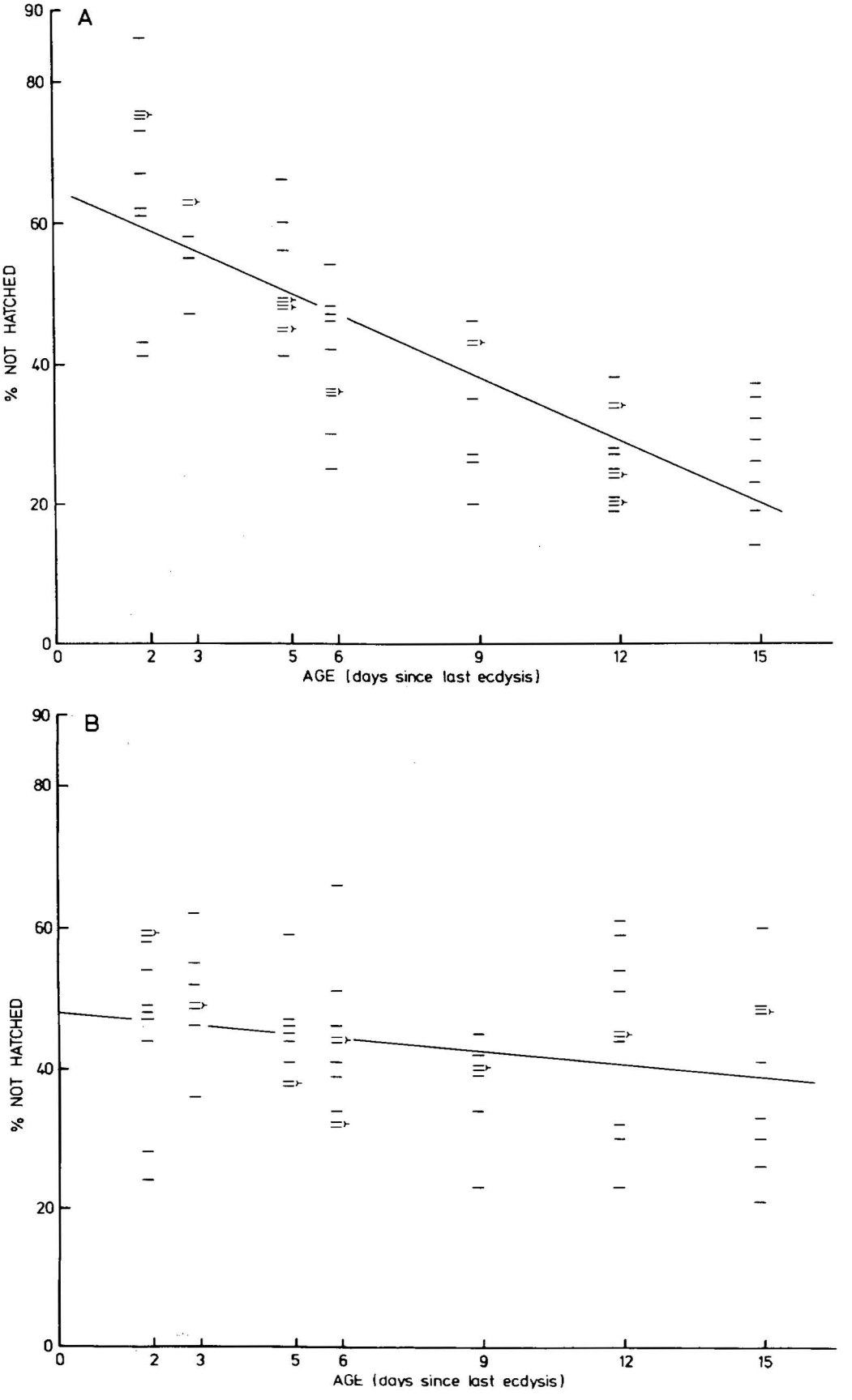


Fig. 1. The relation between age of F_1 females in days after the last ecdysis and the percentage mortality among their eggs: (A) F_1 females from the crossing G♀♀ × R♂♂; (B) F_1 females from reciprocal crossing, R♀♀ × G♂♂. Each point represents the percentage mortality among the eggs collectively produced by ten F_1 females during a 20-hours period. The means of the seven groups of replicates are not indicated but the best fitting straight lines through the means are shown.

this effect is probably absent or unimportant.

F_1 females from the reciprocal crossing do not show the same improvement of fertility with age (Fig. 1B). There is a slight decrease of egg mortality with ageing, which is only moderately significant by Spearman's rank correlation test ($n = 7$, $p \cong 0.05$, two-sided). Young F_1 females of the crossing G♀ × R♂ show a higher egg mortality than those from the reciprocal crossing, but the difference is reversed when they are twelve days old or older. Obviously there is a difference between reciprocal crosses with respect to the rate of decrease of egg mortality with ageing.

Influence of physical circumstances and quality of host plant

This section deals with the question whether external circumstances modify the degree of F_2 egg mortality. To what extent can external factors be responsible for the enormous differences between replicates?

In a first experiment 43 samples of ten F_1 (R♀ × G♂) females of the same age were subdivided at random into five groups and each group was placed in a different climatic room, where they were allowed to oviposit for 18 to 30 hours. Females placed under conditions of lower temperature were given more time to oviposit, so that a sample of between 90 and 120 eggs was obtained from every group of ten females. Circumstances in the five climatic rooms were different primarily with respect to temperature (16°, 19°, 21.5°, 24.5° and 27 °C respectively). Conditions of relative humidity and air currents were less well controlled. The eggs were kept under these conditions until the viable ones had developed well past the larval stage, when the hatchability percentage was scored. Differences between five groups of percentages (Fig. 2) were moderately significant (Kruskal-Wallis one-way analysis of variance by ranks: $p \cong 0.03$). However, there is no clear correlation with temperature. The lowest mortality occurred at the highest temperature (27 °C), but also at 19 °C the mortality is relatively low. Therefore the possibility must be reckoned with that some of the uncontrolled features of the physical environment have a certain influence. Even differences in physical circumstances between different corners of the climatic room may theoretically influence egg mortality. All the experiments reported on hereafter are performed simultaneously in the same climatic room, and leaf cultures carrying egg samples belonging to the same experimental groups are not placed together in one tray but mixed between egg samples of different groups.

There is also the possibility that the quality of the leaf on which the females are feeding influences the hatchability of their eggs. This possibility was checked by dividing 22 leaves into two equal halves by cutting out the midrib. The 22 leaves were chosen so that they contained a great variety of

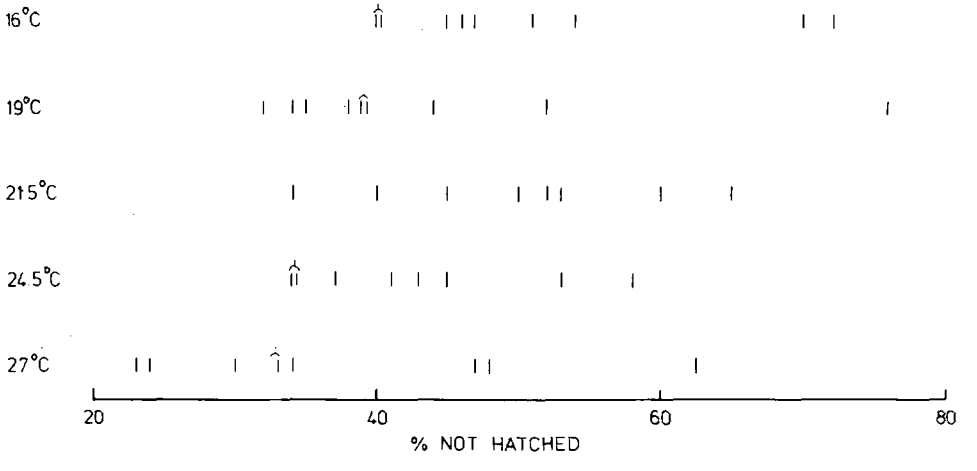
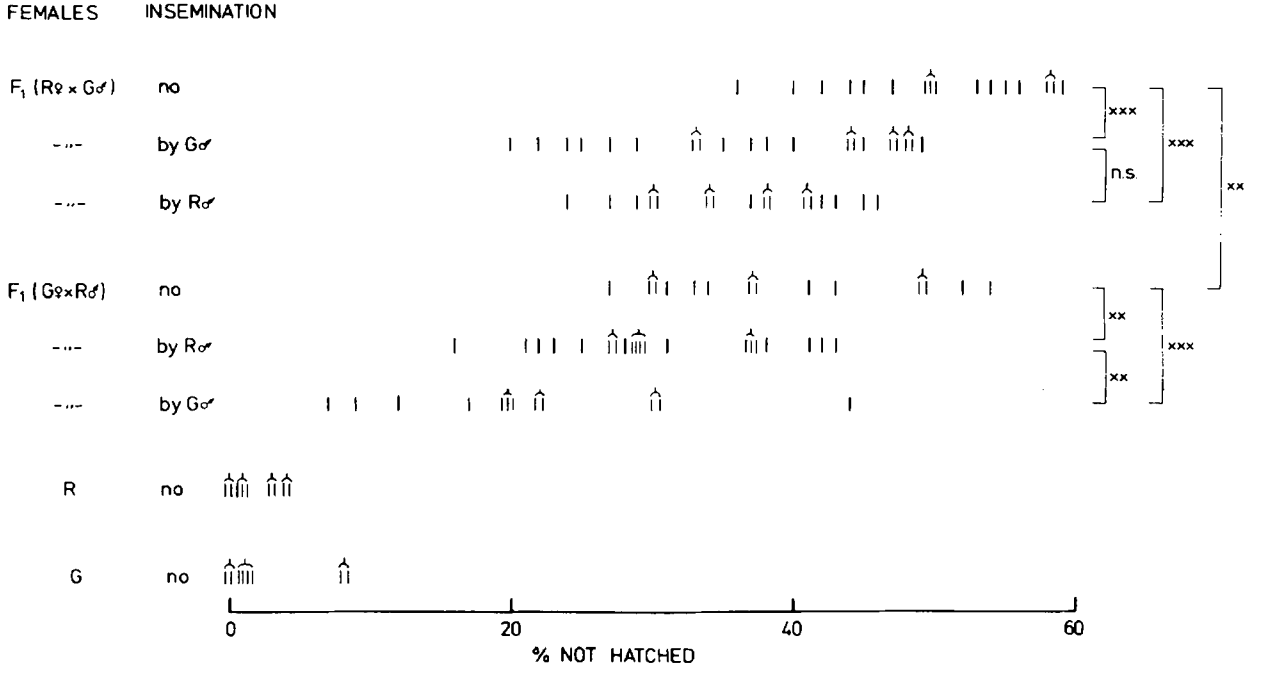


Fig. 2. Mortality percentages in the eggs produced by virgin F_1 (R♀ × G♂) females of the same age placed in climatic rooms with different temperatures. Each percentage concerns the eggs (90 120) collectively produced by ten females during 18 to 30 hours and relative humidity ranged from 70 to 80%.



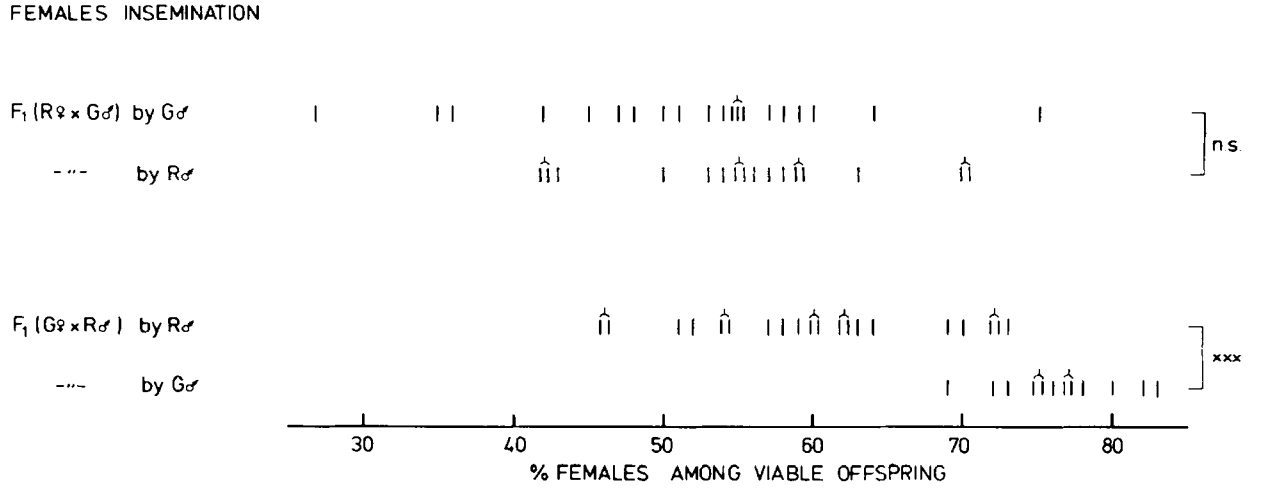


Fig. 3. Degree of non-viability (top) and sex-ratio (bottom) in the progeny of F_1 females of two reciprocal crosses, R♀ × G♂ and G♀ × R♂, and of non-hybrid females of the R and G strain. The females are either uninseminated, inseminated by males of the R strain or inseminated by males of the G strain. Each value concerns the progeny produced collectively by ten females in their seventh day of adult life during on average 20 hrs. The significance of differences between the various classes of values is indicated at the right; n.s.: not significant; *: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $p < 0.001$ (Mann-Whitney U test, two-sided).

ages, colour intensities and vigour. Forty-four leaf cultures were prepared with these leaf halves. On each one, ten F_1 (R♀ × G♂) females of the same age were allowed to oviposit for 20 hours at 27 °C. The resulting 44 egg-mortality values did not show any relation with the identity of the leaf. In fact the between-leaf variance was a little smaller (93.26, d.f. = 21) than the within-leaf variance (106.11, d.f. = 22). Therefore it can be assumed that the quality of the leaf culture on which the eggs are deposited has no influence on egg mortality.

Another possibility is that the quality of the host plants on which the F_1 females have been reared affects the hatchability of their eggs. The results of the experiments described in the next section were used to study this effect. In the last stage of the experimental procedure, F_1 females, reared together on the same leaf culture, were divided into two groups of ten and each group was placed on a separate leaf culture in order to produce egg samples for hatchability assessment. In this way duplicate egg mortality values were obtained. After the removal of the group differences (c.f. next section), the within-duplicate variance was 63.18 (d.f. = 49) and the between-duplicate variance was 71.18 (d.f. = 48), which is not significantly different ($F = 71.18/63.18 = 1.127$, d.f. 1 = 48, d.f. 2 = 49, $p = 0.34$). Therefore it can be concluded that the quality of the host plant on which an F_1 female and her immediate progenitors developed, does not significantly influence the mortality rate of her eggs. Even the between-duplicate variance is insignificant in comparison with the variance between the groups dealt with in the next section ($F = 1297.45/71.18 = 18.22$, d.f. 1 = 5, d.f. 2 = 48, $p \cong 0$).

Differences between reciprocal crosses and the effect of fertilization

Fifty-two samples of ten virgin F_1 (R♀ × G♂) females and forty-six samples of ten F_1 (G♀ × R♂) females, all of the same age, were each subdivided at random into three groups. One group was left unfertilized, one group was mated with males of the R-strain and one group was mated with males of the G strain. Of every group of females one egg sample was collected on the seventh day of adult life for assessment of the number of inviable eggs and the numbers of viable male and female offspring. The results are given in Figure 3, together with the mortality values of similar samples of eggs laid by non-hybrid R and G females. Differences between reciprocal crosses are best studied by comparing the unfertilized eggs. The eggs from virgin F_1 (R♀ × G♂) females show a significantly higher mortality rate than those from virgin F_1 females of the reciprocal cross (Mann-Whitney U test, $p \cong 0.002$, two-sided), although, in conformity with Figures 1A and 1B, they are nearly equal at this age.

Eggs produced by inseminated females show a significantly lower mortality in all cases (Mann-Whitney U test, $p < 0.01$, two-sided). Therefore it can be concluded that a fertilized egg has a better chance to hatch than an unfertilized (haploid) one.

The eggs produced by F_1 (G♀ × R♂) females show a much lower mortality when the female is fertilized by a male of the original maternal strain, G, than by a male of the R strain (Mann-Whitney U test, $p < 0.01$, two-sided). Two not mutually exclusive explanations for this are possible: (1) a higher degree of fertilization is accomplished with

Table 2. Proportions of viable and unviable male and female progeny of six classes of hybrid females (cf. Fig. 3). The proportions of males and females among unviable eggs are estimated (for explanation see text). Values based on estimates are in italics.

Females	Fertilization	Unviable eggs	Progeny				% mortality		
			females		males		among females	among males	Degree of fertilization (%)
			unviable	viable	unviable	viable			
F_1 (G♀ × R♂)	no	814	0	0	814	1253	-	39.4	-
F_1 (G♀ × R♂)	by R males	910	*394*	1228	*516*	795	*24.3*	*39.4*	*55.3*
F_1 (G♀ × R♂)	by G males	402	*166*	1167	*236*	364	*12.5*	*39.4*	*69.0*
F_1 (R♀ × G♂)	no	1069	0	0	1069	1094	-	49.4	-
F_1 (R♀ × G♂)	by G males	1054	*218*	881	*836*	856	*19.8*	*49.4*	*39.4*
F_1 (R♀ × G♂)	by R males	890	*205*	872	*685*	701	*19.0*	*49.4*	*43.7*
R(non-hybrid)	no	18	0	0	18	943	-	1.9	
G(non-hybrid)	no	20	0	0	20	803	-	2.4	-

sperm from the maternal strain (it is very unlikely that any females remained unmated in the presence of males) and/or (2) an egg fertilized by a sperm from the maternal strain has a higher probability to hatch than an egg fertilized by a sperm from the paternal strain. In spider mites the proportion of females in the progeny reflects the degree of fertilization. The F_1 (G♀ × R♂) females inseminated by G males do indeed show a high proportion of viable females in their progeny, which seems to credit the first hypothesis. However, it is not known how many of the unhatched eggs were males and how many were females. These proportions can only be estimated, based on the proportion inviable eggs in samples of purely unfertilized eggs: In a mixture of fertilized and unfertilized eggs the ratio inviable males / viable males, should be equal to the ratio inviable eggs / hatched eggs in a sample consisting of only unfertilized eggs. For the total number of eggs in each of the six classes an estimate is obtained in this way of (1) the percentage mortality among diploid eggs, (2) the percentage mortality among haploid eggs and (3) the degree of fertilization (sex ratio). These values are given in Table 2. Three important observations can be made: (1) in F_1 females of both reciprocal crosses the degree of fertilization is higher when inseminated by males of the original maternal strain. The difference is 13.7% for F_1 (G♀ × R♂) females but only 4.3% for F_1 (R♀ × G♀) females. (2) The mortality among diploid eggs is lower when fertilized by sperm from the original maternal strain. This difference is 11.8% in case of the F_1 (G♀ × R♂) females and only 0.8% in case of the F_1 (R♀ × G♂) females. It seems therefore that differences in degree of fertilization as well as differences in mortality have contributed to the difference in over-all egg mortality accounted for by the type of males provided for insemination. (3) Among fertilized eggs mortality appears to be still considerable. The lowest of the four values, 19%, is much higher than the mortality among non-hybrid haploid eggs (1.9% and 2.4%). Therefore the viability-restoring effect of fertilization is apparently not complete, even in case of fertilization by sperm from the maternal strain. This is in agreement with the findings of Overmeer & Van Zon (1976). The estimates are based on data showing a large quantitative variation, but the agreement with the results of Overmeer & Van Zon make it highly probable that mortality occurs among diploid eggs as well as among haploid eggs, although at a lower rate.

The first backcross generations

Fifty-six samples of ten virgin B_1 females, which were the daughters from either of four types of crosses, F_1 (R♀ × G♂) ♀ × R♂ (14 samples), F_1 (R♀ × G♂) ♀ × G♂ (14 samples), F_1 (G♀ × R♂) ♀ × G♂ (14 samples) and F_1 (G♀ × R♂) ♀ × R♂ (14 samples), were allowed to lay eggs during on average 20 hours in their fourth day of adult life. There is considerable mortality among the eggs laid by these females (Fig. 4). As in the previous section the samples of females belonged to duplicates with identical backgrounds and again after removal of the group differences the between-duplicate variance was not significantly greater than the within-duplicate variance ($F = 172.1/123.0 = 1.40$, d.f. 1 = 27, d.f. 2 = 28, $p = 0.19$). The between-duplicate variance was again very small in comparison with the between-group variance ($F = 937.4/172.1 =$

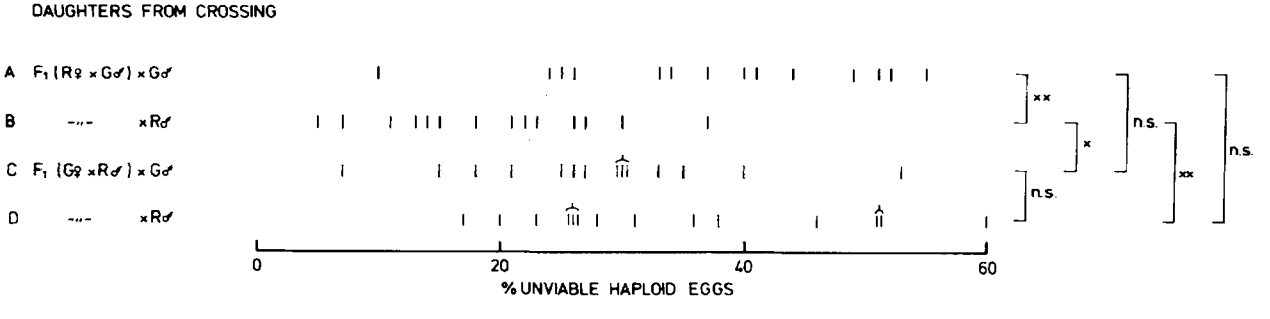


Fig. 4. Mortality in the eggs produced by virgin daughters from any of four types of first backcrosses. Each percentage concerns the eggs produced collectively by ten B_1 females in their fourth day of adult life. The significance of differences between the four groups of values is indicated at the right; n.s.: not significant, x: $0.01 < p < 0.05$, xx: $0.001 < p < 0.01$ (Mann-Whitney U test, two-sided).

5.45, d.f. 1 = 3, d.f. 2 = 27, p = 0.0046).

If the mortality is caused by an interaction between chromosomal factor and maternally inherited (cytoplasmic) factors, the results are in line with what would be expected: The mortality is highest in the eggs from those females who have a majority of their genes from a different strain than their maternally inherited factors (A and D in Fig. 4). It is uncertain, however, if this situation will remain so when the B_1 females get older. The females denoted B and D in Figure 4 should both have about ¼ of their genes from the G strain and ¾ from the R strain. That the egg mortality of these females is nevertheless significantly different may indicate that maternally inherited factors are involved or perhaps a maternal effect extending over more than one generation. However, it should be remembered that these females, as a group, experienced much mortality in their early development. This mortality may have been selective, a different cytoplasm selecting different genes. Therefore the average genic composition of the B and D females may well be different. In other words, a maternal effect extending over no more than one generation could also explain the differences in egg mortality.

Repeated backcrosses

Three strains were started, each with six R females fertilized by G males. Of each strain between six and eight pharate F_1 daughters were collected and isolated with G males. Such backcrosses to G males were repeated during eight consecutive generations. Then two groups of ten virgin B8 females were taken from each strain. One group was provided with males from the G strain, the other with males fromt he R strain. The first crossing gives rise in fact to a B9 generation. The female offspring from the second crossing will be denoted: F_1 (B8♀ × R♂) females. Ten virgin daughters from each one of these crosses were allowed to produce eggs collectively during 20 hours in their fourth day of adult life. The mortality percentages in these eggs are given in Table 3. The mortality percentages in the eggs from B9 females are in the same order of magnitude as those from F_1 females. This result seems to indicate the involvement of maternally inherited factors. Therefore it is a bit surprising that a crossing of B8 females with males from the original maternal strain, R, gives rise to similarly sterile females. In fact the percentages egg mortality of B9 and F_1 (B8♀ × R♂) females of the same strain are remarkably alike in all three cases. In this connection it is of interest that the results of another study (De Boer, unpublished) strongly indicated that the non-Mendelian agents involved in this hybrid infertility are ultimately gene-controlled (see also Discussion).

Table 3. Mortality among the haploid eggs of females derived from three strains. Each strain was started with F_1 (R♀ × G♂) females and propagated by repeated backcrossing to males of the original paternal parent strain, G, for eight generations. Left: daughters from the crossing B8♀ × G♂ (= B9 females); right: daughters from the crossing B8♀ × R♂.

Backcross strains	B9-females		F_1 (B8♀ × R♂) females	
	eggs	% not hatched	eggs	% not hatched
a	136	51	144	55
b	130	37	49	35
c	126	44	97	48

Egg mortality and egg production

A correlation between egg production of F_1 females and the mortality rate among their eggs can be demonstrated if the F_1 females from different combinations of strains are compared. Low egg production is usually accompanied with high egg mortality (De Boer, 1981). This correlation was also demonstrated in crossings between hybrid strains, obtained through large-scale hybridization of the R and G strains (De Boer, unpublished). In view of these facts it is of interest that such a correlation was not present among the replicates of crosses between the (non-hybrid) R and G strains (present study).

Discussion

Differences in fertility between F_1 females, differences between reciprocal crosses and changes in fertility with ageing of the F_1 females, all strongly indicate that non-Mendelian factors are involved in the mechanism giving rise to hybrid infertility

between the R and the G strain. Lack of uniformity regarding fertility of the F_1 females has been reported from studies on other strains as well (Keh, 1952; Boudreaux, 1963; Dosse, 1963; Dosse & Langenscheidt, 1964). Therefore it seems that in general non-Mendelian factors are involved in the F_1 infertility between strains of the *Tetranychus urticae* complex. It also seems to be a general characteristic of this inter-strain sterility, that F_2-egg mortality is partly restored after insemination, more so if the inseminating males belong to the original maternal parent strain than to the original paternal parent strain. (c.f. Overmeer & Van Zon, 1976). Also the fact that F_1 sterility is usually not complete seems to be typical. Cases of complete F_1 sterility have been reported but these may well concern extremes in the range of F_1 infertility. Moreover such F_1 progenies often, if not always, on closer inspection contain a very few F_1 females laying some viable eggs (Boudreaux, 1963; De Boer, 1981). Therefore it may well be that the same or a basically similar mechanism is underlying all these cases of hybrid infertility.

Although there is no doubt that non-Mendelian factors are involved, the results of a different study on the same R and G strain (De Boer, in prep.) make it highly probable that such non-Mendelian factors are ultimately controlled by chromosomal genes: Colonies were established, starting from hybrids between the R and the G strain. After these hybrid strains had been propagated for one year (about 20 generations) by endogamous reproduction, the intra-strain fertility was back to normal. Crossing experiments indicated that the affinities between these strains and the original parent strain are determined by the genes, R or G, that were in excess at the initiation of the strains. Strains that started off with an excess of R genes showed good inter-fertility with each other and the original R strain and bad inter-fertility with the G strain, etc. The ultimate source of the maternally inherited (cytoplasmic) factors proved to be of minor importance. This finding is remarkable in view of the present results with repeated backcrosses: Eight generations of repeated backcrosses to males of the paternal parent strain G, did not yet convert the strain into an intra-fertile G strain, in spite of the fact that in all probability all the factors involved are gene-controlled.

A gene-controlled extra-chromosomal agent is also described in *Drosophila melanogaster* in connection with a kind of hybrid sterility, similar in many respects with the phenomenon dealt with in this paper (Bucheton & Picard, 1978). Sterility is expressed in daughters from a cross between males carrying a chromosomal factor, I, and an extra-chromosomal factor, R (Pélisson & Picard, 1979). The R factor is, however, in the long run controlled by factors localized in the chromosomes (Bucheton & Picard, 1978). Other similarities with the hybrid sterility in spider mites are: (1) the sterility of the F_1 females is not complete; only part of their eggs are inviable and (2) hatchability of the eggs produced by F_1 females improves when these females get older (Bucheton, 1978; Bucheton, 1979). In F_1 (G♀ × R♂) females viability of the eggs appears to be restored by sperm from the maternal strain more than by sperm from the paternal strain. It is based on an inaccurate estimate but the difference is rather big (24.3% and 12.5%). This is in contrast with the F_1 sterility in *Drosophila melanogaster* where the type of male inseminating the F_1 female has no influence on egg mortality (Picard *et al.*, 1977). A remarkable difference with the sterility phenomenon in *D. melanogaster* concerns the distribution of the various strains. In *D. melanogaster* only two types of strains can be distinguished: Inducer (carrying I) and reactive (carrying R). Wild populations seem to belong invariably to the first type, whereas among laboratory strains a substantial part belong to the second type (Picard *et al.*, 1976). This is in striking contrast with the abundance of semi-incompatible strains in the *T. urticae* complex, where hybrid infertility usually comes to expression in both reciprocal crosses, although with quantitative differences.

It is surprising that these phenomena have not been discovered earlier in a species so frequently used for crossing experiments as *Drosophila melanogaster*. It causes one to wonder if this kind of phenomena are not much more widespread in the animal and plant kingdoms.

Tetranychus urticae is also relatively well studied and it is outstandingly suitable for the observation of these phenomena. Crossing experiments are techniqually easy to carry out and dead eggs do not easily escape attention. The arrhenotokous way of sex determination may have facilitated the recognition of this egg mortality since it is expressed especially among haploid eggs. Indeed the same

kind of phenomenon has been described in two other tetranychid mites: *Tetranychus neocaledonicus* (Gutierrez & Van Zon, 1973) and *Panonychus citri* (Inoue, 1972) and one phytoseiid mite, *Typhlodromus occidentalis* (Croft, 1970). The fact that in *Drosophila melanogaster* the phenomenon is found only in laboratory populations is intriguing. It may be presumed that severe isolation of small, genetically impoverished populations is a necessary condition for the development of this kind of strain differences. Spider mite colonies are presumably often founded by a single inseminated female, brought to a vacant habitat by passive airborne dispersal (Boyle, 1975). Gene flow between different field populations also depends on such undirected dispersal. It would be interesting to study the inter-fertility of geographic populations in other organisms with a similarly restricted mobility.

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