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## THE TAXONOMY AND IDENTITY OF PAPILIO NITRA W.H. EDWARDS IN COLORADO (PAPILIONIDAE)

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With APPENDIX by Lee D. Miller

*Papilio nitra* W.H. Edwards and *P. gothica* Remington are members of the *P. machaon* complex of questionable status. *P. nitra* often has been thought to be a hybrid (Clark and Sheppard, 1955; Remington, 1956, 1968), and the validity of *P. gothica* has come under scrutiny more recently (Clark and Sheppard, 1970; Shapiro, 1975). The purpose of this paper is the discussion of these two entities and their relationships to each other in the Front Range of Colorado based upon my breeding experiments during 1972-1974.

Since its description *P. nitra* has been classified as a full species, but more recent authors have expressed the opinion that it might be a hybrid between *P. zelicaon* Lucas and *P. polyxenes asterius* Stoll. Clarke and Sheppard (1955) obtained phenotypes resembling *P. nitra* from laboratory crosses between European *P. machaon* and *P. polyxenes asterius*. These authors state, "It [*nitra*] is very like the hybrids between these two species in all respects," continuing, "found in an area where *P. machaon* [referring to *zelicaon*] and *P.p. asterius* overlap." Scott, Ellis and Eff (1968) note that *P. nitra* "always flies with *zelicaon*, but never with *polyxenes*." It has been my experience that *P. polyxenes* is uncommon or unknown in much of the range of *P. nitra* north of Colorado, but all three occur sympatrically in the foothills west of Denver Northward to the Boulder area.

Remington (1968) described *P. gothica* mostly on the basis of biological and ecological data. He separated *P. gothica* from *zelicaon* on the basis of hybrid differences, especially differences between the sex ratio and progeny of *gothica* x *polyxenes* versus *zelicaon* x *polyxenes* crosses. Misinterpretation of the data produced by these pairings by Remington is explicitly noted by Clarke and Sheppard (1970) who disputed the specific distinctness of *P. gothica*. It should be expected that populations from widely separated localities will differ enough to alter hybrid characters when paired with other stocks. Such evidence, while suggesting some genetic differences, is not enough to prove speciation. Shapiro (1975) disputes the specific distinctness of *P. gothica* because *gothica*-like characteristics appear in spring generation *P. zelicaon* from central California. His results indicate that *P. gothica* may be an environmental form, based on his examination of lowland populations of *P. zelicaon*. It should be noted that these populations, which solely were considered by Shapiro, may be in fact a recently evolved "ecological subspecies" in urban and farming areas of California. At the time *P. zelicaon* was described, little, if any, *Foeniculum*, the larval foodplant of this lowland population occurred

in California. Possibly these lowland, multiple-brooded populations are a result of higher elevation, univoltine individuals invading the disturbed and cultivated areas where *Foeniculum* has become widespread. It therefore might be expected that individuals from lowland areas would possess characters of the higher elevation material, especially since these are closer to the Rocky Mountain populations, at least ecologically.

Clarke and Sheppard (1970) and Shapiro (1975) clearly indicate reasons why *P. gothica* cannot represent a valid species as presented by Remington (1968). Based on my breeding experiments, I believe that the Rocky Mountain populations, here included with *P. zelicaon*, are in some respects different from West Coast *zelicaon* populations. In the following text "*gothica*" is used for the yellow Colorado phenotype of *P. zelicaon* and "*nitra*" for the black phenotype.

## GENETIC EVIDENCE AND DISCUSSION OF DATA

The breeding work began in 1971 when I collected a female *gothica* on Lookout Mountain west of Denver in early June. This female layed nearly 150 mostly fertile ova. The larvae were reared on the natural host plant, *Harbouria trachypleura* (A. Gray). The adults that emerged the following spring are shown in Table 1 demonstrating the phenotypes obtained. Brown (1975) mentioned these preliminary results. An approximate 50:50 ratio of yellow to black phenotypes occurred in the  $F_1$  generation. These were comparable to typical wild-collected specimens of *P. gothica* and *P. nitra* from the area.

$F_1$  sib-matings of *nitra* male and *gothica* female produced highly fertile ova. Individuals again were reared on natural foodplant and produced the  $F_2$  generation, the data for which are summarized in Table 1. The ratio of black to yellow progeny was comparable to that of the  $F_1$ .

TABLE 1

Phenotype	$F_1 \delta - \text{♀}$	$F_2 \delta - \text{♀}$	$F_1 \%$	$F_2 \%$
gothica	33-25	21-31	47	54
nitra	34-32	21-23	53	46

Table 1:  $F_1$  and  $F_2$  adult phenology. The  $F_1$  is the results from the wild-collected yellow *gothica* phenotype of an unknown pairing. The  $F_2$  is the results from a sib pairing of a black *nitra* male and yellow *gothica* female phenotypes.

Other  $F_1$  sib pairings were made using black male and female individuals. The results of these pairings are shown in Table 2. The ova were again very fertile, but under laboratory conditions black females were reluctant to oviposit, resulting in smaller numbers of offspring produced.

Sib-matings were made of  $F_2$  adults, and fertility was much reduced compared to that of  $F_1$  sib pairings, a not unusual happening in inbred stocks. Unfortunately, the entire  $F_3$  generation was accidentally fumigated, and all crosses succumbed to this treatment except a few individuals of a wild male *P. polyxenes asterius* a female of the *nitra* morph. That cross is discussed below.

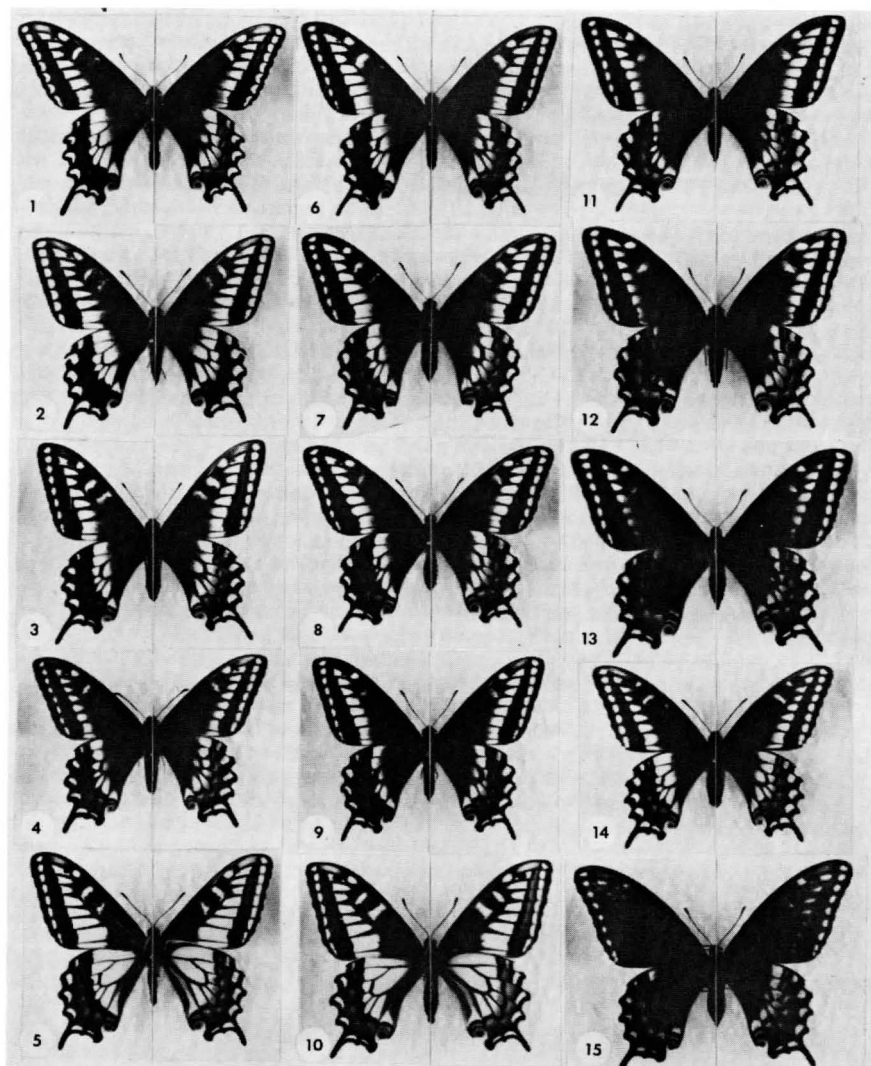
From the data presented in Tables 1 and 2, the following conclusions may be made. If simple complete dominance of the black gene is assumed, as demonstrated by Clarke and Sheppard (1955), the following must be true:

- 1) The father of the  $F_1$  generation was heterozygous for the black gene,

TABLE 2

Phenotype	NxN (1)	NxN (2)	( $\delta - \text{♀}$ )
gothica	7-2	0-6	
nitra	9-7	4-3	

Table 2:  $F_1$  sib pairing of black male and female phenotypes from two separate pairings. The number of adults obtained does not reflect the total number of viable ova nor the mortality of pupae during the diapause stage which was higher in these crosses than those in Table 1.



Figures 1-15: *Papilio zelicaon nitra* (1-13) and its hybrids with *P. polyxenes asterius*. In all figures the left half is the dorsal surface and the right half ventral. All specimens approximately 0.40 x . 1-4, Wild collected males from the Front Range of Colorado; (1), Horsetooth Mountain, 7000' (2135m), Larimer Co., 31 May 1971; (2), Jarre Canyon, 6500' (1982m), Douglas Co., 1 June 1975; (3), Mt. Zion, 6800' (2074m), Jefferson Co., 16 May 1974; (4), Soldier Canyon, 6500' (1982m), Larimer Co. 5, wild collected male of form "gothica"; Fall River Road, NW of Idaho Springs, 7500' (2287m), Clear Creek Co., Colorado. 6-9, F<sub>1</sub> generation *P. zelicaon nitra* females exhibiting approximate range of variation in this brood. 10, Form "gothica" female, reared from wild collected larva; Horsetooth Reservoir, W of Fort Collins, 6000' (1830m), Larimer Co., Colorado. 11-13, F<sub>1</sub> generation *P. zelicaon nitra* females exhibiting approximate range of variation in this brood. 14-15, Progeny of cross of *P. zelicaon nitra* female (F<sub>2</sub>) x *P. polyxenes asterius* male (Colorado Front Range population); 14, male; 15, female.

otherwise yellow progeny could not have been produced. This conclusion is supported by the resulting  $F_2$  generation.

2) The sire of the  $F_2$  generation in Table 1 was also heterozygous, since yellow offspring appeared in a similar proportion as in the  $F_1$  generation. Therefore, all black  $F_2$  individuals could only be heterozygous for that condition.

3) Table 2 shows that the black individuals from the  $F_1$  were certainly heterozygous for the condition, too. Of the 75% black individuals expected in the offspring of such pairings (a 3:1 ratio), two-thirds of these black individuals should have been heterozygous for the trait. An all-black generation would be possible if one of the parents were homozygous for blackness.

Although in nature such homozygous individuals may exist, the collecting data tend to indicate their probability to be nearly zero. The dilution of black genes in the yellow population, representing recessive genes for the condition, no doubt prevents the homozygous black state. This conclusion is supported by several facts. 1) The *gothica* form is commoner than is the *nitra* form in the study area, averaging a ratio of about 8:1 over several years. 2) Since the proportion has remained relatively constant, the percentage of the black gene must be more or less stable. 3) More yellow-yellow matings occur than any other kind, since it is the commonest morph. 4) The mixed pairings that do occur are probably all heterozygous black parents since the phenotypic ratio of the population seems unchanged year to year. Few black-black matings are probable, since that morph is already in short supply in population. 5) Therefore, most black individuals (if not, in fact, all) are the result of heterozygous black specimens mating with yellow ones. Since it is not known how certain modifier genes noted by Clarke and Sheppard (1955) affect the black and yellow genes, it is possible that yellow-yellow matings where one or both parents is itself the result of a black-yellow pairing, could be responsible for maintaining a very low frequency of black individuals in the population. Such modifiers almost certainly account for the variability of band width of the *nitra* found in the study area.

From the breeding experiments it is concluded that *nitra* is not a sterile hybrid derived from the mating of two different species. It is a fully viable insect, conspecific with *gothica*. Fertility of sib-matings was characteristic in all combinations attempted in either  $F_1$  or  $F_2$ . These were as follows: ♀ *gothica* x ♂ *nitra*; ♀ *nitra* x ♂ *gothica*; ♀ *gothica* x ♂ *gothica* and ♀ *nitra* x ♂ *nitra*.

#### THE ORIGIN OF *P. NITRA*

The exact origin of the black *nitra* phenotype is not fully understood and largely is left to speculation. The most probable explanation involves *introgression* of *P. zelicakon* (*gothica* morph) with *P. polyxenes asterius* involving the genes controlling ground color. Introgression is here defined as the incorporation of genes from one species population into another without both parental populations losing their identities. Hybridization of *gothica* populations with *P. p. asterius* may have introduced genes for black phenotypes into the former and have introduced modifier genes for a darker overall pattern. *P. z. "gothica"* is a darker insect than is typical *P. z. zelicakon* and *gothica* x *asterius*  $F_1$  hybrids are darker than are those from *zelicakon* x *asterius* (Remington, 1968). This introgression probably occurred at or during original contact between the two parent entities.

Introgression as a possible explanation for the introduction of black genes into a yellow population is supported by the fact that yellow *gothica* remain the common morph even though the *nitra* trait is dominant. There may be some selection for the black gene in the foothills of the Rockies of Colorado and little or no selection toward this gene in the higher altitudes where *nitra* has not been taken. Perhaps, too, the gene for black never was introduced into the higher elevation demes. Whatever the origin of *nitra*, it is a genetic form of a species, rather than a rare product of continuous hybridization.

Hybridization of *nitra* and *P. p. asterius*, both rather similar insects, and subsequent backcrosses of the offspring into *polyxenes* or *gothica* could account

for the occurrence of oddly fasciated field-collected specimens in the study area and, at the same time, provide an indirect means of maintaining the black genes in the *gothica* population. A wild male *P. p. asterius* was bred to an  $F_2$  female *nitra*, producing viable ova and both sexes of progeny (Figs. 14-15). I consider that present direct, active hybridization of *P. p. asterius* and the *gothica* morph is only a remote occurrence.

#### DISTRIBUTION AND IDENTIFICATION OF *P. NITRA*

*P. nitra* chiefly occurs from Alberta to east-central Colorado, east of the Continental Divide. I have examined one specimen from west-central New Mexico (Mt. Taylor) indicating that it may be found further south than previously thought. No *nitra* are recorded at present between Mt. Taylor and Douglas Co., Colorado, although *gothica* is found there. In Montana and Idaho the only *nitra* from west of the Divide are found. (I have not examined Idaho specimens). The exact distributional limits of *gothica* are yet to be determined, as noted by Remington (1968). It certainly occupies a far greater altitudinal range than does *nitra*, ranging from 6000' (1830m) to above timberline (11,500', 3500m) in Colorado. *P. nitra* occupies the lower part of that range with *gothica* (up to about 8000', 2440m, in Colorado). This is an important link in the relationship between *nitra* and *gothica* because the two share a mutual distribution and occur sympatrically wherever the former is found. No black individuals are known from the type locality of *gothica*, Gothic, Gunnison Co., Colorado, 9000' (2745m).

*P. nitra* is phenotypically variable, especially in the study area. The bred series illustrates this variability well and is comparable to the variation observed in wild collected specimens. *P. nitra* may be confused with *P. p. asterius*. Several characters of *nitra*, when used in conjunction with the figures given here (Figures 1-13), will help distinguish even the most atypically marked specimens:

- 1) The overall wing shape is more characteristic of *P. zelicaon* than *P. p. asterius*. Hybrids of male *polyxenes* x female *nitra* show the females strongly exhibiting the flowing forewing of *polyxenes*; the males are slightly intermediate.

- 2.) The postmedian bands on the male upper surface, while variable in width, always have the spots "fading" proximad into the ground color, since in this respect they are simply overscaled on the black ground color.

- 3) The postmedian band of the under hindwing are most strongly orange (often completely orange) in those spots opposite the discal cell of both sexes. The most anterior spot of the submarginal series is also orange (often also on the upper surface). Other members of these spots may be dusted with orange, but they are never red as in *P. p. asterius*.

- 4) The male usually has a distinct second bar in the cell of the forewing above, although it may be reduced to only a few scales.

- 5) Females, as in *polyxenes* and some other related *Papilio*, exhibit polymorphism for pattern. In addition to the under surface pattern characters mentioned, *nitra* females may be separated from those of *polyxenes* by the well developed postmedian band of the forewings. Occasional specimens may have only the most anterior spots well delineated, with the remainder fading posteriorly to give a tapered appearance to the band, be it wide or narrow. Females from northward in the range of *nitra* and *gothica* frequently have the postmedian band complete on both wings, occasionally as strongly developed as in the males. The strong polymorphism exhibited by the Colorado females is attributed to recurring contact with *P. p. asterius* which is absent further north in the range of *nitra*.

- 6) In both sexes the abdomen is laterally striped with a yellow line broken into a series of elongated dashes. Some specimens may possess a partial second series of spots, but the abdomens are rarely, if ever, marked exactly like those of *P. zelicaon* or *P. p. asterius*.

## SYSTEMATIC RELATIONSHIP AND TAXONOMIC ASSIGNMENT

The breeding data suggest that *P. nitra* is conspecific with the yellow phenotype that Remington described as *P. gothica*. Although the breeding work reported here was confined to the Front Range of Colorado, there is little reason to expect deviation from the general process throughout the rest of the range where *nitra* and *gothica* are recorded. The following taxonomic reorganization of this complex is proposed for *P. zelicaon*, *nitra* and *gothica*:

*P. zelicaon* Lucas, 1852

a. *P. zelicaon zelicaon* Lucas, 1852

= many other synonyms.

b. *P. zelicaon nitra* W. H. Edwards, "1883" (1884)

f. *norm.* "*gothica*" Remington, 1968 [NEW SYNONYMY]

This assumes, of course, that form names are desirable for retention, but in this instance, *gothica* is the much more abundant "normal form" of *nitra*, and the former better shows its relationship to *P. zelicaon* than does *nitra*.

## CONCLUSIONS

Breeding experiments involving *P. gothica* and *P. nitra*, both previously considered separate species, have shown that these two entities are genetically related. Further these conspecific populations have been related to the more western *P. zelicaon* and in fact represent a Rocky Mountain subspecies of *zelicaon*. Introgression of *P. polyxenes asterius* and these *P. zelicaon* populations may have introduced black genes from the former into the latter's gene-pool in the study area, resulting in the constant low frequency of dark morphs of *zelicaon*. Though earlier direct hybridization may have been initially responsible for the black phenotype in *zelicaon*, it now cannot be invoked to completely explain the present situation. Present day crossing in nature may preserve the black genes, or perhaps there are selective pressures working positively in at least the study region.

This study suggests that perhaps similar genetic polymorphism may be responsible for some of the problems in three other complexes within the *P. machaon* group: 1) *P. machaon*, *zelicaon* and *P. kahli* Chermock and Chermock (both Dakotas and adjacent southern Canada); 2) *P. polyxenes rudkini* J. A. Comstock and its forms *clarki* and *comstocki*, both described by Chermock and Chermock (southern California and Arizona) and 3) *P. bairdii* and its forms *brucei* and *hollandi*, all described by W.H. Edwards (southwestern U.S., especially Colorado).

The occurrence of black phenotypes in coastal Californian *P. zelicaon* populations as noted by Emmel in Howe (1975) may represent introgression with *P. bairdii*. They do not fit the concept of Rocky Mountain *nitra* as defined here. I am in the process of studying the genetics of *P. bairdii* in Colorado and invite the collaboration of others working in related areas within the *P. machaon* complex in North America.

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## APPENDIX

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Perhaps there is no such thing as a "pure *nitra*"! Fisher's data seem to indicate the idea that the gene for the "*nitra*" morph (*N*) might be lethal in the homozygous condition and may exist in a balanced polymorphism with the "*gothica*" (*n*) gene. Therefore, all of what the taxonomists call "*nitra*" may be heterozygous (*Nn*) at the locus in question, whereas all of what we call "*gothica*" would be homozygous *nn* at the site. The application of this hypothesis to the problem presented by Fisher might explain (1) the lowered fertility of *Nn* x *Nn* crosses and (2) the observed frequency of the "*nitra*" morphotype and its stability in the study area. This hypothesis in no way contradicts Fisher's careful work, but it does explain the results in a somewhat different manner.

All of the "*nitra*" stocks used in Fisher's experiments were definitely heterozygous *Nn* at the locus in question, and the matings to *nn* individuals produced the expected 1:1 ratio of *Nn* and *nn* individuals. The reduced number of progeny obtained in *Nn* x *Nn* crosses, while partially explicable by the lower vitality of the insects because of inbreeding (comparison of the  $F_1$  vs. the  $F_2$  numbers of offspring), may be explained very satisfactorily by the lethality of an *NN* genotype, probably in the egg stage. Pooled data for the *Nn* x *Nn* crosses do not fit a 3:1 ratio that could be expected by the survival of 1 *NN*: 2 *Nn*: 1 *nn* progeny, and subjections of these data to Chi-square analysis gives a significant deviation from the expected results (Table 1). The hypothesis of a 2:1 ratio of surviving progeny, however, is well supported by statistical analysis of the data. I suspect that the total lowered fertility is explained by the effects *both* of inbreeding and lethality at the *NN* locus.

Perhaps, then, we are dealing with the phenomenon that Cain and Sheppard (1954) call "adaptive polymorphism". In this theory heterozygotes are placed at a selective advantage over either homozygote, and the genetic variability is thus maintained in the population, even when one of the homozygotes is a lethal combination. This could account for the prevalence of low numbers of "*nitra*" in the study area and elsewhere where conditions are more or less comparable. In other, higher elevation areas this heterosis may not obtain, and the *N* gene is selected against, usually strongly, because of its homozygous lethality.

Accordingly, the 8 "*gothica*": 1 "*nitra*" ratio may be explained by a balanced lethal system. Fisher alludes to a possible slight selective advantage for the "*nitra*" phenotype in the study area, and such an advantage may be introduced into the formula with ease (Table 2). The values for gene frequency that fit the observed system are  $N = 0.06$ ,  $n = 0.94$  and the differential fitness components are  $NN = 0$  (lethal),  $Nn = 1$  and  $nn = 0.94$ . These values are only approximate, but with

such a system it is possible to maintain a polymorphism through many generations and not change the observed phenotypic ratio.

Of course, fitness probably changes with time, and there is a possibility that mating behavior might select for the rare genotype, even a lethal one (Bruck, 1957). Neither of these possibilities has been considered here. Nor has the possibility that the "nitra" effect is attributable to a polygenic system: in this case polygenes are deemed less likely since all of the progeny were either "nitra" or "gothica", irrespective of the variability shown in the former. The observed variability can as easily be ascribed to variable penetrance of the *N* gene as to any esoteric polygenic system. Naturally, one would expect modifiers, but the effect of a single gene is capable of producing an approximation to the "ideal nitra".

I suspect, then, that we are dealing with a case of lack of dominance, assuming that the lethality of the *NN* genotype is accepted. This could produce just such an animal population as the one with which we are concerned.

Mathematical details and general population genetic theory are found in Li (1955).

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Table 1: Chi-square analysis of pooled data on "nitra" x "nitra" crosses (see Table 2 in main body of paper). *Nn* refers to "nitra" phenotype, *nn* to the "gothica" phenotype.

	3:1 ratio		2:1 ratio (lethal <i>NN</i> )	
	<i>Nn</i>	<i>nn</i>	<i>Nn</i>	<i>nn</i>
Observed	23	15	23	15
Expected (e)	28.5	9.5	25.33	12.67
Deviation (d)	5.5	5.5	2.33	2.33
d <sup>2</sup>	30.25	30.25	5.429	5.429
d <sup>2</sup> /e	1.061	3.184	10.214	0.428
chi-square (1 degree of freedom)	4.245		0.642	
Probability	.0517		.5017	

\* significant deviation.

Table 2: Mathematical model of selection acting on the study population of *Papilio zelicaon nitra*. *NN* = postulated lethal genotype; *Nn* = "nitra" morph; *nn* = "gothica" morph. Gene frequency arbitrarily set at *N* = 0.06, *n* = 0.94; fitness measurements: *NN* = 0 (lethal), *Nn* = 1, *nn* = 0.94. Random mating is presumed.

	<i>NN</i>	<i>Nn</i>	<i>nn</i>
before selection	0.0036	0.1128	0.8836
fitness	0	1	0.94
after selection	0	0.1128	0.8306
proportions of new population	0	0.1196	0.8804
new gene frequency	<i>N</i> = 0.0598		<i>n</i> = 0.9402

Succeeding generations may be calculated similarly.