THE GENETICS AND REPRODUCTIVE ISOLATING MECHANISMS OF THE *PIERIS NAPI - BRYONIÆ* GROUP

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I. Introduction

The case of *Pieris napi* L. and *bryoniæ* Ochsenheimer has wasted much ink in arguments among taxonomists as to whether *bryoniæ* is a distinct species or only a subspecies of *napi,* without having brought about a satisfactory explanation. Even after the extensive work of Muller and Kautz (1938), a turning-point in the opinions on this problem, the situation remained rather unchanged for a more critical or biologically trained judge, familiar to some extent with the circumstances in this difficult group. This failure is mainly due to the fact that most early investigators as well as Muller and Kautz themselves have not used the genetic approach. The research of B. Petersen (1948-1955) offered a first refreshing exception, and his important conclusions anticipate some of those reached in the present paper. Nevertheless a more exhaustive genetic analysis of the *napi - bryoniæ* complex remained necessary. In fact, at the same time as I was about to publish my genetic research obtained some years ago, several articles of Bowden appeared (1955, 1956, 1957, 1958) containing also some genetical and experimental data about the questions involved, though a conclusive genetic survey has not been given. Besides this, his results differ in some respects so much from my own that it will be by no means unnecessary to show here the results of my investigations in the usual genetic way.

The Problem. The main attractive point for the taxonomist lies in the great color differences between the females of these two forms as well as in the geographic restriction of *bryoniæ* to the Alps, Carpathians, Scandinavia, and to some less known parts of Asia and North America. On the other hand, *napi* is widely distributed in both the plains and the mountains of the Palæarctic and Nearctic Regions but usually does not appear in the areas of *bryoniæ.* Because of its restricted boreal - alpine distribution and the occurrence of more or less common transitions towards *napi, bryoniæ* was in the past considered to be an ecological race of *P. napi.* However since Muller and Kautz have in their extensive work offered certain arguments in favor of the specific distinctness of the two forms, their view has gained more and more adherents among lepidopterists, so that finaly Forster and Wohlfart in their work *Die Schmetterlinge Mitteleuropas* (1955) quoted *bryoniæ* as a good species,

although the problem has not reached its conclusive stage. My use here of the term "adherents" suggests that we are not concerned with a systematically simple case, but rather with one that is in some respect ambigous. Such cases of controversial opinions have much interest from the evolutionary point of view, because this fact itself suggests that we are dealing with an incomplete stage of speciation, which deserves a closer analysis, especially from the genetical and populational aspect.

The present paper refers to my research concerning the genetics of the morphological characters of *napi* and *bryoniæ*, to the occurrence of recombinations of these characters in nature, to the possible and experimental hybridisation and to the degrees of reproductive isolating mechanisms between the two forms.

II. GENETICS OF COLOR DIFFERENCES BETWEEN *P. bryoniæ* AND *P. napi*

MATERIALS AND BREEDING METHODS. The crossings were performed with the ssp. *neobryoniæ* Sheljuzhko that is abundant in the Alpine parts of western Yugoslavia and with *napi* from the lowland districts of the environments of Zagreb, where no *bryoniæ* occur. Some crossings were also obtained with Alpine *napi* captured in districts where they fly together with *bryoniæ* (Krnica Valley, Rateče, Planica). During 1947 - 1951 about 200 broods were reared to imagos and more than twice as many matings have been carried out. Previously (1926, 1931, 1954) broods from wild females occasionally captured at the lower situated localities of *bryoniæ* (Fala, Savinja valley, Krnica below Prisojnik) served rather as an orientation for further decisive research.

The matings were obtained always under the experimentator's control either inside the laboratory, mostly at the windows, or outdoors in the net, sometimes also free in the field. Many pairings could be realised only by the authors' artificial copulatory method using clamped females and immobilised males (Lorković 1948, 1952). The mating usually lasted 1 to 1½ hours. The egg-laying females were kept in cages provided with a cotton net and the eggs were deposited on the potted food plant *Roripa silvestris*, where the larvæ remained until the last molt. Subsequently the larvæ were transported to plants kept in water. The plants must be changed every one or two days whether they are eaten by the larvæ or not. By this way the losses caused by diseases were reduced practically to zero. The pupation occurred on the walls of the cage or in cardboard boxes, where the larvæ were placed after they had stopped feeding. Emerged male imagos usually were kept in cardboard boxes about 15 × 15 × 30 cm for two or three days at a lower temperature (15°

- 18°C) wherefrom they were liberated once daily for some minutes for feeding. In such a manner the males can be kept in good condition before they become ripe to mate; this usually takes two or three days before one may with great probability count upon the mating ability of a male. On the contrary, the females are able to mate as soon as their wings grow strong enough for flying. Older females are more convenient only in the case when the artificial pairing methods must be used.

GENES AFFECTING THE WING COLOUR OF *bryoniæ* AND *napi*. The first clear and doubtless assertion about the inheritance of the main *bryoniæ* character, i. e. the dark melanic markings along the wing veins in *bryoniæ* females (these are missing in *napi*) has been expressed in PETERSEN'S paper (1955), where on the strength of my unpublished research he reported that the *bryoniæ* character is dominant over that of *napi*. A short note appeared previously to the paper mentioned in the *Proceedings of the IX. International Entomological Congress* (1952), where I declared in the discussion of HESSELBARTH'S report that the *bryoniæ* pattern is dominant, whereas the inheritance of the brownish yellow color is intermediary. All other previous opinions about this matter, so far as is known to me, were uncertain, which is no wonder, for without reared F_2 generation or back-crosses such a decision could not be reached. In fact, nearly all previous breeders, as well as some recent ones, suffered great losses caused by diseases, owing to the faulty breeding methods, so that the poor F_2 broods were not sufficient for an accurate genetical analysis. One exception was MAIN'S crossing between 1907 and 1909, but in spite of a satisfactory breeding method he reached a quite erroneous conviction "that the inheritance of *bryoniæ* characters passed almost entirely through the female" (quoted after BOWDEN, 1956). After this failure it was not earlier than in 1956 that BOWDEN published the first successful research on this matter, and his results agree in general with my own. In respect to the lacks in the genetic interpretation of the crossings in his paper I shall give here a genetical analysis of my crossings in connection with the question of the reproductive isolation of these two butterflies.

The following crosses have been carried out by the author:

	napi ♂ × *bryoniæ* ♀	BROOD
1. 1932:	♂ Podsused (Zagreb); ♀ Rogovilec, Savinja valley, Karawanken Alps	R × P
2. 1947:	♂ Maksimir (Zagreb); ♀ Vršič, Julian Alps, 1400 m.	III
3. 1948:	♂ Maksimir (Zagreb); ♀ Vršič, *ex larva* 1947	1 bn

bryoniæ ♂ × *napi* ♀

4. 1935:	♂ Mangart, Julian Alps; ♀ Podsused (Zagreb)	M × P
5. 1948:	♂ Vršič, *ex larva* 1947; ♀ Peričnik, Vrata valley, Julian Alps, 900 m.	3 nb
6. 1948:	♂ Krnica, Julian Alps, 1100 m.; ♀ Mt. Sljeme (Zagreb), 600 m.	(27)5

Besides the listed crossings, several *bryoniæ* broods from various localities of the Julian Alps and the Karawanken Alps in the Yugoslav part of the southeastern Alps have been carried out, *i. e.* from: Žirovnica, 700 m.; Fala near Maribor, 300 m.; Rogovilec in Savinja valley, 650 m.; Vrata valley in the Julian Alps, 800 m.; Vršič and Mojstrovka in the Julian Alps, 1400-1800 m.; and Bovec in the Trenta valley, 600 m. *P. napi* were mostly used directly from nature, since *napi* is the species of the environment of Zagreb, ranging eastwards to the line Maribor - Zidani most approximately, where the last small populations of a light *bryoniæ* can be found.

The most important genetic results were obtained from the cross "III" which could be brought up to the 14th generation without introduction of any new *bryoniæ* blood, but with 7 refreshments of outdoor *napi*.

The recent analysis of these crossings confirmed the author's previously mentioned remarks about the inheritance of the main *bryoniæ* and *napi* characters. The three taxonomic distinctions between these two forms are controlled by three independent pairs or groups of genes: 1) An autosomal gene pair controls the extension of the dark MELANIC COLOR along the wing-veins; its dominant allele, *B*, causes the *bryoniæ* females to be heavily infuscated along the veins, whereas with the recessive allele, *b*, characteristic for *napi*, the darkening along the veins is either absent or reduced to the outer (distal) part of the veins (Fig.1 ♀ ♀). In the male sex the manifestation of these alleles is only slight, it is similar in both forms and therefore markedly sex-controlled. 2) The BROWNISH-YELLOW GROUND COLOR of *bryoniæ* females, and the WHITE one of *napi* controlled by another pair or group of genes, *Y*, *y*, and its expression is strongly sex-controlled, since the males are completely white. 3) A third pair of alleles, *W*, *w*, affects the WHITE or GREENISH-YELLOW GROUND COLOR of the underside of the hindwings and the apex of the forewings, especially in males, and is thus partially sex-controlled.

We shall now turn to the more detailed analysis of each of the mentioned gene-pairs.

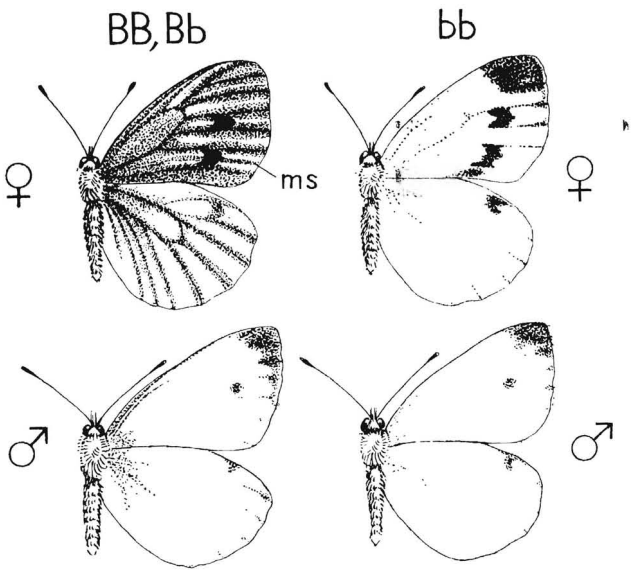

Fig. 1. Melanic wing color pattern in females and males of *bryoniæ* (left) and *napi* (right) which are controlled by the allele pair *B* and *b*.

THE *B*, *b* ALLELES. The main controversy concerning the allelic pair *Bb* which controls the principal feature of *bryoniæ* against *napi* is the question of its dominance. Because of the fact that the *bryoniæ* × *napi* hybrids show a less pronounced *bryoniæ* pattern than the pure *bryoniæ* it was believed earlier that this character behaves intermediately. Such opinions rest on a misinterpretation of the meaning of dominance. This term does not denote full identity with the parental phenotype, respectively the homozygous one, since there are all transitions between the complete dominance to none at all. In our case, by pairing homozygous *bryoniæ* and *napi*, the F_1 generation of both reciprocal crossings is always of the *bryoniæ* pattern, although a somewhat slighter one. In the F_2 or any later generation, when individuals heterozygous for the genes *Bb* are mated, *bryoniæ* and pure *napi* segregate in a 3 : 1 ratio. In backcrosses the segregation is likewise clear, and the ratio is 1 : 1. In Fig.2 the succession of 14 generations obtained in the stock "III" is represented diagrammatically. The genotypes of each brood or of the parents as

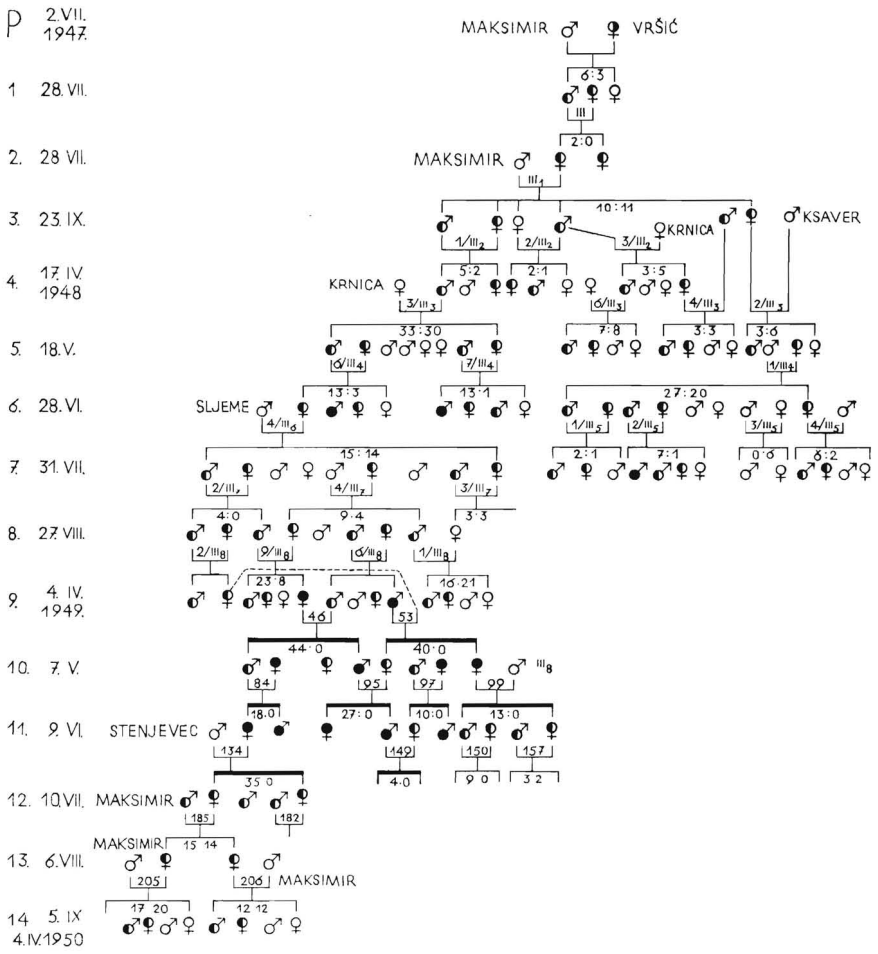

Fig. 2. Diagrammatic representation of 14 successive generations in the stock "III" of the *napi* ♂ × *bryoniæ* ♀ crossing. During this time only *napi* were introduced from outside. The actual ratio of phenotypes is indicated for each brood; the expected genotypes are indicated by black (*BB*), black and white (*Bb*) and white (*bb*). Male and female symbols show approximately the given ratio. The bold black lines indicate broods in which all individuals were of *bryoniæ* phenotype, consequently at least half of them were homozygous.

indicated in the diagram result from actual ratios obtained in the progeny of each brood as well as by the symbols of male and female phenotypes. The four possible ratios which occurred in 54 broods are summarized in Tables 1 – 3, each brood containing not less than 15 individuals; broods with a lower number of descendants are not included, except a few which are of special interest.

Table 1. *Bb* × *Bb*. Expected 3 : 1 ratio.

Brood	Ratio						χ^2
	B♂	*b*♂	*B*♀	*b*♀	*B*♂♀	*b*♂♀	
M×P – 1935	14	2	8	3	23	5	0.762
III – 1947	2	0	4	3	6	3	0.333
$6/III_4$ – 1948	7	0	6	3	13	3	0.333
2/4b – 1948	13	2	6	4	19	6	0.013
9 – 1948	12	3	11	5	23	8	0.011
128 – 1949	10	2	2	1	12	3	0.200
201 – 1949	11	3	22	5	33	8	0.657
214 – 1949	11	5	12	1	23	6	0.287
247 – 1950	12	4	11	3	23	7	0.044
265 – 1950	15	6	14	6	29	12	0.202
289 – 1950	21	8	4	1	25	9	0.039
301 – 1951	15	3	10	5	25	8	0.010
307 – 1951	11	4	4	2	15	6	0.142
5bn – 1948	3	2	3	3	6	5	2.455
Total:	157	45	117	45	274	90	0.015
Expected ratio:	151.5	50.5	121.5	40.5	273	91	

Table 2.

A. *BB* × *BB*, *BB* × *Bb*, *BB* × *bb*. Expected 1 : 0 ratio.		B. *bb* × *bb*. Expected 0 : 1 ratio.	
Brood	Ratio	Brood	Ratio
46 - 1949	44 : 0		0 : 14
53 - 1949	40 : 0	$3/III_5$	0 : 6
73 - 1949	16 : 0		0 : 20
45 - 1949	27 : 0	14/2/4b	0 : 17
127 - 1949	29 : 0	213 - 1949	0 : 15
134 - 1949	35 : 0	235 - 1950	0 : 17
167 - 1949	11 : 0	235 - 1950	0 : 11
210 - 1949	58 : 0		
231 - 1950	28 : 0		
237 - 1950	18 : 0		
241 - 1950	68 : 0		
271 - 1950	22 : 0		
- 1950	37 : 0		
315 - 1951	26 : 0		

Table 3. ♂ *Bb* × ♀ *bb, and* ♀ *Bb* × ♂ *bb.* Expected 1 : 1 ratio.

Brood	Ratio						χ^2	Sex of *bb* parent
	B♂ : *b*♂		*B*♀ : *b*♀		*B*♂♀ : *b*♂♀			
RxP$_1$-1932	11	10	28	30	39	40	0.068	♂[1]
RxF$_3$-1932	—	—	11	12	11	12	0.010	♂
1/III-1947	7	4	3	7	10	11	0.046	♂
3/III$_3$-1948	21	13	12	17	33	30	0.142	♀
6/III$_3$-1948	2	5	5	3	7	8	0.066	♀
1/III$_4$-1948	13	12	14	8	27	20	1.043	♂[2]
4/III$_6$6-1948	8	10	7	4	15	14	0.035	♂
bbn-1948	4	5	3	2	7	7	0.000	♂[3]
165-1949	4	3	3	3	7	6	0.077	♀
185-1949	4	9	11	5	15	14	0.035	♂
205-1949	12	8	5	12	17	20	0.242	♂
206-1949	5	6	7	6	12	12	0.000	♂
236-1950	3	3	3	4	6	7	0.076	♂
239-1950	13	11	8	12	21	23	0.091	♂
264-1950	6	6	3	4	9	10	0.052	♀
278-1950	7	10	8	6	15	16	0.032	♀
284-1950	8	3	5	8	13	11	0.666	♂
312-1951	13	5	6	8	19	13	1,125	♂
313-1951	10	6	4	7	14	13	0,036	♂
Expected ratio:	151	129	146	158	297	287	0.171	
Total:	140	140	152	152	292	292	0.50>P<0.70	

[1]The greater part of young larvæ was abandoned.
[2]Several *napi* females were accidentaly lost.
[3]*bryoniæ* ♂ *ex larva* from Vršić, 1947.

The Chi-squared test and the probabilities (P) of the ratios 3 : 1 and 1 : 1 convincingly prove that the difference between the *bryoniæ* and *napi* pattern depends on only one single pair of alleles and that the *bryoniæ* pattern is dominant over that of *napi*. Accordingly, the *napi* individuals are always homozygous, *bb*, and consequently pure-breeding; *bryoniæ* can be either homozygous or heterozygous. It can be seen from the diagram of Fig.2 that the mother of the stock "III" was heterozygous, since her progeny segregated in 6 *bryoniæ* and 3 *napi*, a quite unexpected fact for a high-alpine *bryoniæ*, usually considered to be true-breeding. It must be pointed out that in all these broods a clear-cut discontinuity between the *bryoniæ* and *napi* patterns was absolute, although *bryoniæ* vary considerably. The best but by no means the only decisive diagnostic character which allows the clear distinction between bearers of *B* and *b* has been found in the so called "margin-streak" ("Saumstrich" of MULLER

and KAUTZ or BOWDEN's "bryo-streak"), *i. e.* the dark suffusion along the rudimentary vein A_1, extending from the posterior discal spot to the outer wing margin (Fig.1, ms). This streak is so rarely to be found in *napi*, that when it is present it is more than probable that it originates from a previous mixture with *bryoniæ*. It is present nearly always not only in each similarly patterned subspecies or species as *adalwinda* Fruhst., *bicolorata* Petersen, *pseudobryoniæ* Vrty., *camtschadalis* Röb., and *ochsenheimeri* Stgr., but in *Pieris melete* Mén. too. Spring *napi* females which show a more marked gray suffusion along the veins than is usual in this species are also distinguishable by the lack of this streak. The distinction between a slightly marked heterozygous *bryoniæ* female and a more strongly marked *napi* would be sometimes difficult without the bryo-streak present in the former and absent in the latter. In general, there is a good correlation between the vein markings and the bryo-streak in *bryoniæ*, the streak being a more reliable diagnostic character inasmuch as it is a qualitative one. One exception to this rule will be considered below. It may be also emphasized that the occurrence of the bryo-streak does not depend upon the presence of the posterior discoidal spot, since the streak can be well developed even when the spot is completely missing.

Despite the fact that *napi* were three times introduced from outside into the stock "III" during the first six generations (not counting the parental crossing), no particular diminution of the *bryoniæ* pattern could be noticed. On the contrary the homozygous *BB* individuals which appeared first in the 10th generation were obviously darker and had broader vein markings than the mother. This is also proof that the mother was, in spite of her high-alpine origin, heterozygous.

In spite of the well-proven monohybrid inheritance of the *bryoniæ* - *napi* pattern one could hardly explain the extreme range of variability of *bryoniæ* by only a twofold nature of its genotype. Moreover, the same phenotype can be either homozygous or heterozygous; crosses of *napi* with heavily dark *bryoniæ* females give rise to hybrids which do not differ from specimens usually considered as normal *bryoniæ* (brood 3/III-3), while on the other hand several stocks of homozygous *BB* individuals bred from heterozygous parents show a less pronounced *bryoniæ* pattern than the just mentioned primary hybrids. In respect to the clear monohybrid segregation there can be little doubt that this very variable shading of the *bryoniæ* pattern depends on polygenic factors independent of the *B* gene. This is most clearly shown by the observation that among the progeny of the two similarly heterozygous *bryoniæ* sisters but with different *napi* fathers (back-crosses), both phenotypes

bryoniæ and *napi* may be considerably different in the dark shading, depending on the intensity of the dark pattern of the *napi* father mated to the *bryoniæ* mother (brood 278). If the *napi* father has a dark pattern, the entire progeny which segregates into *bryoniæ* and *napi*, will have a dark one, too. Similarly a paler *napi* father gives rise to brighter individuals of both forms, *bryoniæ* and *napi* (Fig. 3). Although the shading varies in both crosses, the *bryoniæ* pattern is preserved, since even in the palest females the bryo-streak is fully developed. Such pale females can always be reliably distinguished from every *napi* individual. One can compare the differences described to more or less exposed or developed copies made from the same photograph negative.

Thus, the intensity of this *bryoniæ* character depends not only on the *B* gene itself but also on the genes which control the appearence of melanin in general. Apparently we have here an analogy to the expression of the dark markings in the Nun Moth, *Lymantria monacha*, as it was postulated by GOLDSCHMIDT (1921, 1928).

Apart from such multiple chromogene genes there are also other genes which control the extent of the dark suffusion along the veins. It is well known that this suffusion varies from very narrow lines, hardly broader than the veins themselves, to such an extent that the dusky suffusion of two neighboring veins joins together and the whole surface of the wings becomes dusted with dark scales. A good support for the opinion that this suffusion depends on separate genes has been obtained in a cross between a heterozygous ♂ *bryoniæ* × *napi* and a ♀ *Pieris ergane* female, reared in 1935. The dark pattern of *P. ergane* is identical to that of *P. napi* or *P. rapæ;* but similarly to *P. manni* the spots have less-defined contours and the outer part of the forewings is often largely dusted with dark scales. In the female hybrids (*bryoniæ* × *napi*) ♂ × *ergane* ♀ the dark markings along the veins are strikingly broader on both the forewings and the hindwings than was the case in the father's (*bryoniæ* × *napi*) sisters, that show the width of the suffusion usual for *bryoniæ* × *napi* hybrids.

Consequently, the expression of the dark melanic pattern in *bryoniæ* would be dependent upon three independent groups of alleles: 1) the dominant allede *B* controls the extension of melanin along the veins, and its recessive allele *b* causes the absence of melanin; 2) an unknown number of additively acting genes (*M, m*) control the intensity of the darkening; whereas 3) a probably low number of genes would be responsible for the width of the vein-marking.

Now, after having got acquainted with all the possible genes influencing the dark pattern of *bryoniæ* females, we again must turn to the question of the dominance of the gene *B*. BOWDEN (1956) was right

in his doubt about the dominance of this gene so far as some mixed lowland *bryoniæ - napi* populations are concerned. Instead of a clear-cut distinction between the presence and absence of the bryo-streak, as was found to be the rule in our experiments, here its variability produces all transitions ranging from a common dark streak to a few scarcely visible dark scales or perhaps none. The same seems to apply also to the dark vein markings, which can be as narrow as in some more strongly marked *napi* specimens of pure *napi* populations. In such cases one cannot decide whether such a transitional specimen carries only two *b* genes or also a *B* gene. In my last crossing experiments no such specimens were available, and the crossings carried out many years ago were not complete. However, by a more thorough genetical investigation of this character perhaps very interesting detections could be done. Apparently, two possible explanations may be taken into consideration: either there exist two or even more multiple *B* genes with different manifestation of the bryo-streak, or the recessive gene *b* of these populations is not identical with the gene *b* of the pure *napi* populations, bringing about a stronger vein suffusion than commonly found in *napi,* so that sometimes a slight bryo-streak appears too. Such a supposition could lead to the conception that the gene *b* in *bryoniæ* populations is not derived from hybridization with *napi* populations, but that it takes part in the specific gene pool of *Pieris bryoniæ* as a distinct entity. The confirmation of such a supposition might turn our opinion in favour of the conviction of those authors who consider *bryoniæ* as a separate species. However, in the absence of experimental evidence for any of these hypotheses we must for the present emphasize the genetic proof that the *napi* pattern segregates from heterozygous *bryoniæ.*

Finally, it should be remembered that the intensity of the melanic color depends also upon environmental factors, especially on temperature and humidity. It is known that low temperature and high humidity lead in Pieridæ to spread of melanin, so that it is more than probable that the form "concolor" Röb. is at least partially due to these environmental influences. Pupæ kept in the refrigerator at the time of development gave imagos with extremly wide vein suffusion, while conversely, heat and dryness gave rise to pale and bright individuals.

Everything said so far applies only to the female sex. We must, however, also take into consideration the male sex, although it is not so important for our purposes, since here the phenotypic differences of the genes in question are only slight. Careless observers or novices mostly think that there are no differences between the males of *bryoniæ* and *napi,* which may be true only exceptionally for the spring brood. In the

summer broods *bryoniæ* males are distinguished by gray streaks along the veins on the upperside, particularly on the hindwings, where these markings gradually diminish from the margin of the wing to half way toward the discocellular vein. Rarely the darkening is of such a width that the neighboring markings join near to the wing margin. Usually the markings are developed only as narrow "vein-streaks" as shown in fig. 1, left. In *napi* these gray vein-streaks are either absent or are present as minute dark triangles at the ends of the veins (fig.1, right). In the spring brood the triangles can be prolonged about one millimeter or two at most; this is the only case when males of *bryoniæ* cannot be distinguished with certainty from the *napi* ones.

The vein-streaks are present in every *bryoniæ* male which carries at least one *B* gene, *i. e.* not only in homozygous but in heterozygous individuals too. This circumstance is of great value in the establishment of more precise ratios of phenotypes in segregation, because it is possible to determine the presence or absence of the gene *B* in almost every brood in males as well as females, so that the numbers usable in determining ratios are twice as large. The phenotypic differences of this character correspond in almost every case very well to the expected ratios of 3 : 1 or 1 : 1 (see Tables 1 and 3).

Of course, the stronger vein darking in *bryoniæ* males is present in the forewings as well as the hindwings, although in these the difference from *napi* is not so apparent, because the apical spot always sends shorter or longer vein-streaks towards the inside of the wing.

The demonstration that gene *B*, which controls the dark pattern of *bryoniæ*, is dominant over the recessive *napi* gene, so that pure *napi* traits segregate from heterozygous *bryoniæ*, is of decisive significance. It reveals that *napi* must be always homozygous recessive, *bb*, while phenotypic *bryoniæ* can be either homozygous, *BB*, or heterozygous, *Bb* (Table 2). MULLER and KAUTZ (1938) considered specimens with *bryoniæ* pattern as pure *bryoniæ* or as its ssp. *flavescens* because they did not know this. They also did not know or underestimated the fact that from heterozygous *bryoniæ*, although very dark, specimens of the *napi* phenotype segregate which do not differ from individuals of a pure *napi* population. MULLER even quoted from the literature some cases of the appearence of *napi* specimens within *bryoniæ* broods, but he explained them as individuals accidentaly introduced with the food (*l.c.*: pp.36, 37). Corresponding examples in their monograph are: Tab.6, fig.15; tab.5, fig.11; but also 6, 7, and 8, except for their yellow color. It is true that MULLER and KAUTZ count such *napi* individuals as *bryoniæ* ssp. *flavescens* Wagner, but there is no proof whether they belong to this subspecies or

rather are descendants of a cross with *napi*. It is only certain that such *napi* individuals belong to the *population* of Mödling, which is composed of both *flavescens* and *napi*. Whether they belong to *napi* or *flavescens* cannot be resolved without breedings or crossings, as will be shown below. We shall learn also that *bryoniæ* × *napi* heterozygotes are sometimes present but concealed by the dominance of the gene *B* even in the so called "pure" mountain *bryoniæ* populations.

THE *Y y* ALLELES. It was mentioned above that the factor for the brownish-yellow ground color of the *bryoniæ* females is dominant, although it is not sure that it is really so, since this color shows graduated intensity in both the crossings and in nature. Owing to its strong sex-controlled manifestation it is not easy to decide whether this character depends upon one or more pairs of alleles. Since the males are entirely white, without any trace of a yellowish color, the choice of males in crossings is entirely by chance when pure stocks are not at one's disposal.

The back-cross (R × P - 1932) between an intense yellow *bryoniæ Bb* female from Rogovilec (see below) and a pure *napi* male from Zagreb, where no yellow specimens occurred, seem to be a rather decisive one: 28 yellow and 30 white females appeared, the yellow specimens being represented by all transitions from nearly the same yellow color as the mother had to the very pale one; all white females had the same white color. This cross indicates that the gene for the yellow color is likely to be incompletely dominant, *Y*, its expression being variable, and that there is no more than one allelic pair for this color, a rather unexpected result, since such a gradual color variability usually is attributed to polygeny. However, the characters which are dependent on the cumulative action of polygeny can by no means result in a ratio of 1 : 1, providing, of course, that the estimation of color was correct.

The genetics of this character thus seems to remain somewhat obscure, too.

THE *W, w* PAIR. Especially interesting is the allele W which controls the white color of the underside of the hindwings and the apex of the forewings in the males of *bryoniæ* populations. It is completely dominant over the greenish-yellow color of the recessive gene *w*. The same applies to the female sex, although the gene *w* is manifested only exceptional by the white color, mostly by a pale buff one. The phenotypic manifestation of the recessive allele, *w*, is always the same in males and in females, in *bryoniæ* and in *napi*. *W* is a remarkable gene in that male sex-controlled polymorphism is very rare in Lepidoptera, as pointed out by REMINGTON

Table 4. *Ww* × *ww*. Expected 1 : 1 ratio.

Brood	*W* ♂ : *w* ♂	*W* ♀ : *w* ♀	*W* ♂ ♀ : *w* ♂ ♀	χ^2	P in %
1 bn	13 : 12	17 : 17	30 : 29	0.0169	>80, <90
5 bn	2 : 3	2 . 4	4 : 7	0.8182	>30, <50
4 b	7 : 7	11 : 6	18 : 13	8.8064	>30, <50
4/4b	4 : 0	3 : 5	7 : 5	0.3333	>50, <70
2 b	6 : 6	3 : 3	9 : 9	0.0000	100
24b	13 : 2	5 : 5	18 : 7	4.8400	>2, <5
14-2/4b	2 : 10	2 : 1	4 : 11	3.2666	>5, <10
1 bbn	4 : 0	1 : 1	5 : 1	2.6666	>10, <20
3 bbn	4 : 5	2 : 3	6 : 8	0.2857	>50, <70
6/4b	3 : 0	2 : 3	5 : 3	0.5000	>30, <50
5(27)	5 : 5	— —	5 : 5	0.0000	100
5_2	9 : 9	9 : 10	18 : 19	0.0270	>80, <90
85	0 : 3	4 : 3	4 : 6	0.4000	>50, <70
60	2 : 1	2 : 0	4 : 1	0.9000	>10, <20
Total	74 : 63	63 : 61	137 : 124	0.6475	>30, <50

(1954). Although we do not deal here with a quite true sex-controlled dimorphism, since the difference appears in both sexes, the dimorphism is much more pronounced in the males than in the females, the latter being sometimes difficult to distinguish in respect to it.

It is worth nothing that the genetical identity of the white males and pale buff females has not been realized before the present analysis. Each of these two forms has been proviously considered to be a separate variant: ab. ♂ "subtalba" Schima and ab. ♀ "subtochracea" Kautz. MULLER and KAUTZ reported that "subtalba" can also rarely be found in males and females of *napi*, and they quote the find of a "subtalba" female in Pommerania in northern Germany. It is obvious that the white color of this female has nothing to do with the gene *W*, as was already suspected by MULLER (1938).

Another interesting point of this gene pair is that the author has not as yet been able to obtain a brood homozygous for the gene *W*. As is shown in Table 4, the segregation is the rule and the ratio 1 : 1 prevails. Of the two 3 : 1 ratios, broods 2/4b and 1/bbn, the first is only apparently of this kind since the brood was raised from a back-cross, the mother of the second brood unfortunately escaped before her phenotype was noted. It is striking that 8 of 11 matings between *Ww* individuals were entirely sterile, whereas 3 others gave a total of only 5 individuals, 3 of them in one brood. Only the back-crosses were successful, and even

among these a great part produced no progeny. The writer is inclined to attribute this failure to inbreeding rather than to any other essential cause, except the F_1 hybrid sterility, which will be discussed below.

No population of *bryoniæ* is known in which the gene *W* is the exclusive allele. One of the most significant concentrations of this gene so far known to me occurs in the Julian Alps, where about 45% of all phenotypes are *W* bearers. According to PETER's (1950) count the proportion of the "subtalba" males in the Allgäuer Alps is 19%. No white males had been recorded in the western Alps, as some authors have claimed.

RECOMBINATION IN REARED POPULATIONS. The three gene pairs controlling the morphological features of *bryoniæ* and *napi,* already discussed above, have been found to segregate and completely recombine in the F_2 generation and back-crosses. The dihybrid back-cross "R × P - 1932" already mentioned is especially illustrative. It yielded four female phenotypes: *bryoniæ* pattern and yellow color (*Bb Yy*), *bryoniæ* pattern and white color (*Bb yy*), *napi* pattern and yellow color (*bb Yy*), and *napi* pattern and white color (*bbyy*) in the ratio 14 : 14 : 16 : 14, which agrees very well with the expected dihybrid ratio 1 : 1 : 1 : 1. Besides these two gene groups free combinations with the *Ww* alleles have also been obtained, among them the formerly scantily-known combinations *bYW* and *byW*. Thus the three pairs of alleles are located in different chromosomes, all autosomes. This is only to be expected where the number of chromosomes is so large ($n = 25$). The recombinations are the same as certain "varieties" or "aberrations" from natural collections, already described, and it seems likely that the genetical explanation of these natural variants has therefore been found. I shall mention some examples of such natural "aberrations": *bryoniæ* "obscura albida" (*BB yy*), *bryoniæ* "albida" Müll. (*Bbyy*), *bryoniæ* "flavida reducta" Müll. (*bbYY* or *bbYy*), and *bryoniæ* "albida reducta" Müll. (*bb yy*). This shows at best how unsound it may be to give names to forms which are no more than recombinations of a few gene pairs.

However, before starting with the comparison of the experimental genetic results and the circumstances in nature we must briefly get acquainted with the other morphological as well as physiological and ecological differences between *bryoniæ* and *napi.*

(To be continued)