Egg-laying and Habitat Selection in some *Pieris* Species.

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Animal distribution in nature is to a certain extent dependent on the stimuli emitted by different habitats. By means of these stimuli individuals are sometimes attracted, sometimes repelled. The effect of the stimulus is dependent on the state and activity of the animal and therefore changes during its life. In this paper I have treated some observations made in the Alps 1954 on egg-laying in certain *Pieris* species and its influence on habitat selection. Mainly studied were *Pieris napi* and *bryoniae* and some populations intermediate between these species.[1] Some observations were also made on *P. rapae, manni,* and *brassicae.*

Habitat selection in sympatric sibling species is of special interest. Such species have only recently become ecologically so differentiated as to be able to live sympatrically. As long as two species prefer the same ecological niche they are unable to live together (cf. Park 1954 and literature cited there). Habitat selection and habitat differentiation may be due to quite different factors in different groups. Factors influencing the egg-laying female are, however, of rather general importance. It may be noted that such a differentiation has been shown even in a very simple and homogeneous environment. In food cups placed in population cages containing *Drosophila melanogaster* and *Dr. simulans* both species find the edges of the cups the most desirable place to lay eggs. In addition *Dr. simulans* deposits eggs in the centre more readily than *melanogaster* (Moore 1952).

[1] The intermediate nature of these populations has previously been denied (Müller and Kautz 1938). The strong variation within the populations, from typical *napi* to typical *bryoniae,* confirms this conclusion beyond doubt. Another proof is that typical *napi* and heterozygote individuals previously called *bryoniae* are among the offspring from one and the same female.

Food plants of the larvae.

Pieris bryoniae.—The one-brooded *bryoniae*, which is the only form which really seems to deserve this name, lives on *Biscutella laevigata*. The various records of other food plants mainly refer to the hybrid populations ("double-brooded *bryoniae*"). However, a clear distinction has not always been made in the literature between the food plants of single-brooded and double-brooded forms. This is in fact not easy, for there are transitional populations of all degrees. It is therefore at present not possible to say whether the single-brooded *bryoniae* lives on plants other than *Biscutella*. This plant lives only on calciferous ground, and the distribution and ecology of *P. bryoniae* in non-calciferous areas of the Alps would therefore seem well worthwile studying. In captivity *P. bryoniae* readily lays on several cruciferous plants and the possibility of a similar behaviour in nature cannot be excluded.

A strong preference for *Biscutella* is, however, undoubtedly present. In the Allgäu in southern Germany *Biscutella* grows in a small area of some tens of square meters near Bruck at 810—820 m above sea level. At this altitude *napi* lives normally in this area. Though the spot is situated several kilometres from the nearest *bryoniae* locality, *bryoniae* was found here in 1953 and 1954, imagines as well as many eggs on *Biscutella*. As several other cruciferous species live in the vicinity, the few *bryoniae* females of the area would hardly have found this small locality of *Biscutella* without a special preference for this plant.

The hybrid populations.—In calciferous areas *Biscutella* is an important source of food for the hybrid populations near Vienna and in the Austrian and Yugoslavian Karawanken. Other plants are *Alliaria officinalis* (Kautz 1936), *Thlaspi alpinum* (Müller and Kautz 1938) and *perfoliatum*, and *Arabis turrita* at Mödling near Vienna, as well as *Arabis Halleri* in the area west of Lago Maggiore (Monte Mottarone, Pogallo).

Pieris napi lays on many cruciferous plants, including *Biscutella* (Durmitor area, Professor Lorković, personal communication). Verity mentions for Italy: *Brassica, Rapa, Erysimum officinale, Raphanus raphanistrum, Cardamine amara* and *pratensis, Diplotaxis tenuifolia* and also *Reseda*. To these genera may be added *Armoracia, Barbaraea, Sinapis, Sisymbrium* and *Alliaria*. Müller and Kautz (1938) mention *Arum maculatum* as a subsidiary food.

Pieris rapae lays on various cruciferous plants, particularly often on the cultivated ones with thicker leaves, but also on *Reseda*.

Egg-laying.

The activity of flying butterfly females is mainly directed towards two things: food and plants for egg-laying. Flight during search for

food and during egg-laying is quite different. The amplitude of the wing strokes during the egg-laying flight is greater than normally. At the same time the wings are kept so that the animal is moving forward very slowly, sometimes not at all. As long as this type of flight is going on the females are not so easily disturbed and therefore quite easy to watch. By contrast, they are extremely ready for escape during feeding-flight, feeding, basking, and when they have just gone to rest.

Odours.—During the egg-laying flight the females are attracted by odours from plants on which they lay their eggs. Orientation by the aid of odours is, however, not very good and the appropriate plant is found rather by trial and error. The odour stimulates the female to alight on a plant, usually a leaf. Its nature is then tested while the animal makes a few tapping movements on the leaf with the forelegs. Wrong orientation, close to the correct plant, has been observed 8 to 10 times in succession.

The eggs (except for *brassicae* usually one, sometimes 2—4) are then deposited on the underside of the leaf (cf. figures in Tinbergen and co-workers 1942, Petersen and Tenow 1954). *P. rapae* and *brassicae* lay sometimes also on the upper side. In captivity eggs are often laid on stem, flowers, and fruits, in nature also on fruits, at least those of *Biscutella*.

Light.—The first observations on the influence of light on the choice of plant for egg-laying were made in a hybrid population at Posavje near Ljubljana in Yugoslavia on May 26 and 27. This was already near the end of the flying time of the first generation and only a few animals were still flying. Single females could therefore quite easily be watched as long as their flight did not become too rapid.

The habitat of the population is a mixed coniferous and deciduous forest of several acres. The part where egg-laying was observed is dominated by *Pinus silvestris* in sparse clumps (cf. Fig. 1). Outside the forest are wide meadows with rows of trees (cf. Fig. 2). Under the pines as well as in the open *Biscutella* is common, as seen on both figures. The behaviour during egg-laying is most simply shown by a summary of the records when a female was watched from 12.25 to 13.58 p.m.; temp. 29° C.

12.27—12.30: sunshine; 5 eggs are laid on *Biscutella* growing in the shade of pines.

12.31—12.41: clouds; visits 12 flowers in the forest; 12.39 out into the fields, visits 2 flowers.

12.41—13.05: drizzling rain; visits 22 flowers alternating with rest.

13.05—13.21: clouds; visits 15 flowers

13.21: clouds suddenly thinner; butterfly returns directly to the forest, a distance of almost 100 m, through a hedge, and is after half a minute less than 5 m from the spot where it had laid its eggs one hour earlier.

Fig. 1. Open pine-wood at Posavje near Ljubljana with flowering *Biscutella* in the foreground.

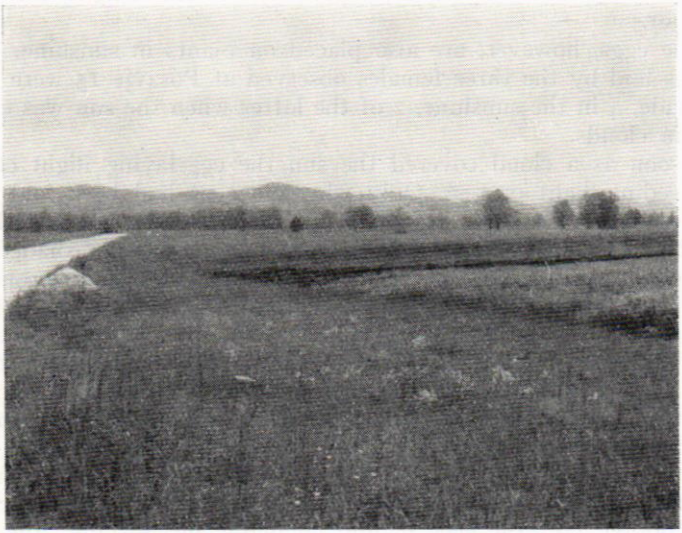

Fig. 2. Fields at Posavje near Ljubljana with flowering *Biscutella* in the foreground.

13.22: thin clouds; "basking", egg-laying flight.
13.25: thin clouds; one egg on *Biscutella* growing in the shade.
13.29—13.32: thin clouds; 2 eggs on Biscutella growing in "sunshine".
13.34: thin clouds; visits a flower.
13.36—13.50: basking position, though the sun was covered with thick clouds.
13.50—13.56: drizzling rain; basking; 2 flights of a couple of metres.
13.56—13.58: drizzling rain; flutters around a *Salix* tree and goes definitely to rest 3.5—4 m above the ground.

Several of the observations made on this female are characteristic of the behaviour of the butterflies observed. Hybrid females as well as *napi* females of the southern Alps predominantly lay their eggs on plants growing in the shade. This shade may be given by rocks, trees, bushes, ferns and other high and dense vascular plants. Now and then the egg-laying flight is interrupted by a few seconds of basking on a leaf, a flower, or on the ground. In this way the eggs are deposited almost exclusively when the sun is shining, and mostly on places in the shade but close to insolated areas. Egg-laying during cloudy weather, as recorded above, is quite abnormal.

Always when the egg-laying females crossed an open place more than a few metres broad, the egg-laying flight changed into flight of the normal type. Sometimes the egg-laying flight was directed towards dense shrubbery where no cruciferous plants were growing. Orientation at such a time obviously occurred by the aid of light rather than of odour.

Some eggs, however, are also placed on plants in sunshine. Of the 19 eggs laid by the three females observed at Posavje 15 were laid in the shade, 4 in the sunshine, 2 of the latter when the sun was covered by thin clouds.

As soon as a cloud covered the sun the egg-laying flight changed into feeding-flight or rest. Only when no eggs had been laid for 56 minutes were a couple of eggs laid, although the sun was not shining.

Hybrid females seem to show a greater preference for shaded areas for egg-laying than do those of *Pieris napi*. At Monte Mottarone both forms were found together (first generation of hybrids, second of *napi*, coming from the valley). Due to the rather steep terrain protracted observations of flying single females were impossible. The few observations made are, however, in agreement with such a conclusion. Its correctness is corroborated by the existence of some hybrid populations in dense forests.

The tendency to lay in the shade is enhanced by high temperature. This fact may explain, at least partly, why *P. napi*, around Stockholm and Uppsala, lays the eggs mainly on plants in sunshine. Egg-laying females of *P. bryoniae* of the Iseler in the Allgäu (1400–1700 m) prefer

open areas to those densely covered with small trees of *Pinus mugo*. By counting the eggs laid in the relatively open areas on plants in sunshine and shade in the middle of the day no preference for plants of any kind could be shown; in sunshine, 66 eggs were found on 7 plants while 20 had no eggs; in the shade, 70 eggs were found on 11 plants and 14 hade no eggs. The eggs were mostly deposited on plants to which the flying females had had free access, and plants growing in sunshine were more often than others covered by grass. The largest number of eggs (together 50–60) were, however, found on two plants (not recorded above) in the deep shade of a *Pinus mugo* and covered by high grass so that the females had had to creep down to lay their eggs.

Thus the light factor during egg-laying contributes but slightly to the habitat differentiation between *P. napi* and its hybrid populations with *P. bryoniae*. This factor is of greater importance in differentiating *P. napi* and *P. rapae* in areas with a high temperature. The latter species deposits the eggs predominantly on plants in sunshine and, even at high temperature, sometimes on the upper side of the leafs.

The differentiation of habitat and behaviour of the two species is strikingly shown by the following two examples:

Along a road at Fiesole near Florence (28.6., 29° C) the area on the southern side was shaded by a 3–4 m high wall while the other side was in the sun. *P. napi, rapae,* and also *manni* were present in the area, the first species predominantly in the shade of the wall, the two latter in the sunshine on the other side of the road. In both areas egg-laying was observed.

At Pallanza (Lago Maggiore area) *P. napi* is common along the shore of the lake while *P. rapae* dominates in the inner part of the town. Here, however, light is hardly the most important factor.

Rate of egg-laying.—At Posavje six hybrid females were observed for 137 minutes. If the type of weather is considered, the number of eggs laid are distributed in the following way:

38 minutes of sunshine	16 eggs
13 minutes of thin clouds	3 eggs
44 minutes of thick clouds	no eggs
32 minutes of drizzling rain	no eggs

Three of the females were observed seven minutes in sunshine during a time of the day when probably they had not yet started their egg-laying activity. Under optimal conditions the females at Posavje obviously lay their eggs at a rate of about one every second minute. During 31 minutes of sunshine the three females spent 79 per cent of their time in a rather constant egg-laying flight.

On Monte Mottarone (1400 m) two hybrid females laid five eggs during 36 minutes, two *napi* females five eggs during 13 minutes. These

data suggest a lower rate of egg-laying on Mottarone than at Posavje, which is in agreement with the less typical and persistent egg-laying flight near the top of Mottarone. Since this type of flight was very pronounced during hot days in the lower parts of the mountain, the difference is obviously explained, at least partly, by the temperature. Whether *Biscutella* (at Posavje) is more easily found by the females than is *Arabis Halleri* (on Mottarone) is not known.

Daily rhythm.

The observations on daily rhythm are rather few and are mentioned here only because so little is known about it in butterflies.

At Posavje the first egg-laying flight was observed as late as 11.56 a.m. Three females were observed before this time, one in the field, two in the forest. All flew rapidly and could be watched only 1–4 minutes. After 11.56 three females were watched for 6, 31, and 93 minutes. All three butterflies laid eggs and the first two were lost, not because of their too rapid flight, but rather during the counting of the eggs laid.

At Monte Mottarone a hybrid female was watched from 8.40 to 10.13 a.m. when the first egg-laying flight began. The weather was optimal with sunshine and 21.7° C at 9.46 a.m. Until 10.10 four shorter flights of together seven metres were made. At 10.00 one egg was laid without any preceding egg-laying flight. The next female in such a flight was not seen until 10.30. Very little egg-laying activity was thus going on before 10 a.m. Its maximum was reached between eleven and one; almost all females finished before 3 p.m., some already before two. A daily egg-laying period of four to five hours thus seems to be an appropriate estimation.

Sleep.

The behaviour of overnighting in trees is characteristic of *P. bryoniae* and hybrid populations. In the same places the pairs sit during the copula. Some observations made on Monte Mottarone throw some light upon the development of this behaviour. The locality is rather steeply sloping towards the north-east. After half past one the shadows of the trees are getting long and the butterflies begin to fly up into the trees for basking. This behaviour is present in *P. napi* as well as in the hybrids of this locality. Once up in the trees it is natural to stay there until the next morning. In so doing the animals have a better chance of being reached by the sunrays and therefore of starting basking earlier. A similar behaviour is advantageous also in a dense vegetation of *Pinus mugo* or *nigra* on more horizontal ground, habitats where this has in fact also been noticed (e.g. Mödling). It is interesting to

note that the same behaviour is retained at Posavje in a hybrid population living in a less dense vegetation. *Pieris napi* from such habitats has not been reported to behave in this manner.

Territorial behaviour.

At Posavje some males and two females were observed visiting flowers in the open meadow outside the pine forest In these meadows *Biscutella* grows, but due to the preference for plants growing in the shade during egg-laying, this is not likely to take place in the meadows. The butterfly whose behaviour is described on pp. 196 and 198 returned immediately and directly to the forest for egg-laying as soon as the clouds covering the sun became thinner (in fact a couple of seconds before I myself had become aware that the weather was clearing up). This fact suggests a sense of direction to the optimal habitat for egg-laying. Without such a sense the sparse hybrid population would be completely dispersed on the wide meadows during feeding—which is not the case. This certainly increases the probability of such a sense of direction in these butterflies.

Discussion.

Mayr (1954) has pointed out, mainly from his experience with birds, that speciation often occurs as a budding-off of geographically isolated populations at the periphery of the geographic range of widespread species. This picture fits very well for many groups of Lepidoptera. Two such widespread species are the closely related *Pieris napi* and *rapae*, while *P. manni* is a peripheral species of the *rapae* group. *P. bryoniae* is now a mountain species entirely surrounded by populations of *P. napi* to which it is most closely related. During the last glaciation it may well have been a peripheral species: Forms similar to *P. bryoniae* are present in such different localities as Ireland, Scotland, Scandinavian mountains, the Carpathians, the Caucasus, mountains of Central Asia, northeastern Siberia, Alaska, and Labrador.

The two present widespread species are, in agreement with conditions found by Mayr (l.c.), more variable at least as far as larval food is concerned. *P. bryoniae* lives only on *Biscutella*, *P. manni* in central Italy only on *Diplotaxis tenuifolia* (Verity 1947). In France it also feeds on some other wild cruciferous plants such as *Lepidium*, *Iberis* and even *Reseda*. This species is, however, never found on cultivated plants such as *Brassica* etc. Adapted to cultivated plants are especially *P. brassicae* (a less closely related species) and *rapae*, but also *napi*.

In the area investigated, the Alps, *P. manni* is very rare. Competition among the other *Pieris* species is, in the larval stage, reduced to a certain extent by a differentiation in the choice of plant species for

egg-laying. However, this competition, as in the case of the *Drosophila* species previously mentioned, is never totally absent. Except for *bryoniae*, which lives on a single plant species, the forms can be placed in the following order according to adaptation to plants with thick leaves and at the same time to cultivated cruciferous plants: hybrid populations *P. napi* × *bryoniae*, *P. napi*, *P. rapae*, and *P. brassicae*.

A similar succession—hybrid populations, *napi*, *rapae*—is also formed if light preference during egg-laying is considered. The differences between hybrid populations and *P. napi* are in this case less striking.

The attraction of some forms to shaded areas during egg-laying is, however, of importance not only for reduction of food competition. In combination with a relatively short daily period of egg-laying in the hybrid females the final effect will be that more eggs and larvae will live in the shade during the hottest part of the day than would happen by mere chance. The temperature preferendum of larvae of *P. brassicae* is 33° (Herter 1953) while some preliminary experiments have given about 28° both for *P. napi* and F_1-hybrids *napi* × *bryoniae*.

Thick leaves in plants are generally considered an adaptation to dry and hot conditions. Species laying their eggs on shaded plants are therefore more likely to attach them to plants with thin leaves. However, as shown by conditions in Scandinavia the two factors, thickness of leaves and light conditions during egg-laying, are to a certain degree independent. Here *P. napi* and *rapae* both lay their eggs mainly on plants in sunshine. Contrary to conditions in Italy *P. napi* is superior in most open habitats, especially in the first generation of the year, but also later. Only on cultivated plants with thicker leaves is *P. rapae* commoner.

Females of the genus *Pieris* lay their eggs on very few and insignificant species outside the family *Cruciferae*. This is certainly the most important factor preventing the genus from penetrating in greater abundance into some deciduous forests of the Alps where cruciferous plants are quite rare. The comprehensive ecological differentiation which has taken place within the genus has, however, enabled forms of the genus to enter most other habitats of the area from sea level to 1800–2200 m. They are extremely abundant in open, cultivated areas but also in many coniferous forests.

In studies of the influence of plants upon speciation and evolution in Lepidoptera, it is mainly their role as food for the larvae that has been considered (W. Petersen 1931, Dethier 1954). As far as habitat selection is concerned—a factor of great importance for evolution—the influence of plants on the egg-laying females is of even greater value. The order of magnitude of this factor compared with others is, however, still unknown.

On the other hand, habitat selection is not only a factor important for evolution but is also guided by natural selection, i.e. evolution.

The two equal sequences of forms—according to the choice of light conditions and thickness of leaves made by the egg-laying female—are quite natural from this point of view. In a harmoniously stabilized ecosystem no other correlation between the two sequences is possible.

Summary.

Two factors are investigated which separate *Pieris* species ecologically to reduce interspecific competition: the nature of the plant species to which the females attach their eggs, and the position of the plants in relation to sunshine.

For both factors the following forms constitute a series: hybrid populations *P. napi* × *bryoniae* — *P. napi* — *P. rapae*. The form mentioned first prefers non-cultivated cruciferous plants with thin leaves which grow in the shade. The eggs of *P. rapae* are most frequently deposited on plants with thick leaves which grow in the open sunshine. *P. bryoniae* is an alpine species which has specialized on a single cruciferous plant. No preference for plants growing in shade or sunshine could be demonstrated in this species.

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