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Phenotypic induction in *Pieris napi* L.: role of temperature and photoperiod in a coastal California population

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ABSTRACT. 1. Californian *Pieris napi* have previously been reported as producing dark-veined and light-veined adults from diapausing and non-diapausing pupae respectively; the converse has not been reported.

2. A population of the coastal subspecies *venosa* from Monterey County was examined to determine whether pupal temperature exposure was involved in proximate control of seasonal phenotypes.

3. When reared under continuous light at 25°C this stock produced seventeen diapause pupae, thirteen of which have eclosed yielding typical dark-veined adults, and thirty-eight non-diapause pupae.

4. Of fifteen non-diapause pupae held in a dark box at 25°C, fourteen produced typical light-veined adults and one produced a dark-veined individual of intermediate phenotype.

5. Of twenty-three non-diapause pupae held in a dark box at 10°C for 24 days, twenty-two produced dark-veined butterflies of intermediate phenotype and one produced a light-veined adult.

6. When the experiment was repeated with an inland population of subspecies *microstriata*, which is normally univoltine and monophenic, the temperature effect on phenotype was still present but less pronounced.

7. The nature of phenotypic determination is reviewed with particular regard for the developmental environment of butterflies from diapaused pupae. The thermal environment of reactivating diapausers, rather than the diapause state itself, may determine adult phenotype.

Introduction

In recent years substantial progress has been made in understanding the polyphenisms of multivoltine butterflies at both the ecological and the physiological levels (Shapiro, 1976). The most significant event in the modern history of such studies was the realization that photoperiod was frequently – perhaps usually – the critical environmental variable for mid-latitude species (Ae, 1957; Müller, 1955, 1956). Although a few instances of direct temperature determination are known (e.g. *Lycaena phlaeas* L., Sakai & Masaki, 1965), all of the classical experiments with European and North American butterflies carried out by Weismann, Standfuss, W. H. Edwards and others at the turn of the century had to be re-evaluated in light of their failure to control or even to describe the rearing photoperiod.

A particularly perturbing case was that of *Pieris napi napi* L. (Pieridae). Both Merrifield (1893) and Weismann (1896) attempted to generate the seasonal polyphenism of this insect by chilling the pupae. Their results were not conclusive, but did imply that dark-veined, i.e. vernal-phenotype, animals could

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be obtained from chilled non-diapause pupae. Oliver (1970) used various temperature treatments on *P.napi oleracea* Harris from New England but was unable to obtain an unequivocal response. He concluded that 'regulation of seasonal dimorphism is thus controlled by larval photoperiod exposure, which also controls the induction of pupal diapause ... it may be that *P.n.napi* has a rather different system for the regulation of seasonal forms from that of *P.n.oleracea*'. In our own studies with Californian *P.n.venosa* Scudder and *P.n.microstriata* Comstock we have observed a reliable correlation of adult phenotype with developmental status (diapause or direct) (Shapiro, 1975).

Weismann's variable results with *P.napi* and the Nymphalid *Araschnia levana* L. led him to theorize that phenotype in these insects was only partly under environmental control, some individuals being genetically determined as one form or the other and therefore refractory to environmental manipulation. Recent work on *A.levana* in Germany (Müller & Reinhardt, 1969; Reinhardt, 1969, 1971) clarified the situation in that species by establishing that under some circumstances temperature treatment could modify or override photoperiodic determination. Was the same true in *P.napi*? An opportunity to investigate arose on 6 March 1976 when Mr S. R. Sims of my laboratory obtained thirty-two specimens from a large population of the coastal Californian subspecies *P.n.venosa* some 6.4 km west of Arroyo Seco camp, Monterey County. The sample contained ten live females, four of which oviposited freely; their ova were pooled for the experiment. All the wild specimens were heavily marked, similar to dark-veined animals from previously-studied *venosa* populations in Monterey and San Mateo Counties (Shapiro, 1975).

Materials and Methods

About sixty ova were obtained. Rearing methods were as described in Shapiro (1975) except that *Brassica campestris* L. rather than *B.kaber* (DC.) Wheeler was used as host. The entire brood was reared at 25°C under continuous light. Since all broods of Californian *P.napi* apparently contain diapause pupae and these cannot be distinguished from non-diapause pupae by inspection, the fifty-five pupae were arbitrarily divided into two lots for treatment. Developmental rates were fairly uniform, and runts were discarded. As individuals pupated they were assigned to one lot or the other alternately, except that five extra individuals were added to the lot to be chilled, to compensate for anticipated mortality. Twenty-five pupae were placed when less than 1 day old in an opaque, heavy cardboard box and held at 25°C. Thirty were placed in a similar box and held at 10°C for 24 days – an attempt to duplicate the Weismann–Merrifield treatments. Both lots were checked daily at approximately 09.00 hours, with about 2 min exposure to light each day. Non-diapausing pupae held at 25°C eclosed in 6–9 days. Refrigerated non-diapause pupae eclosed in 5–9 days after removal to 25°C. Pupae which had not begun to lay down adult pigment after 14 days at 25°C were assumed to be in diapause and were placed in a dark box at 3°C. These pupae were not checked again for 10 weeks, and were checked weekly thereafter.

Results with *P.n.venosa*

Fifteen pupae yielded adults directly at 25°C. All but one of these (i.e. seven males, seven females) were typical light-veined phenotypes, with only a few dark scales along the wing-veins beneath (males) or none at all (females). One female, the last to emerge and a partial cripple, had dark scaling on all the veins of the hindwing beneath and dark points at the vein-tips above. She was lighter than the average dark-veined female of *venosa*, but as dark as the average dark-veined female of the lighter interior subspecies *microstriata*. The remaining ten pupae were assumed to be in diapause, and were refrigerated at 3°C.

Twenty-three of the thirty pupae chilled for 24 days eclosed directly when removed to 25°C. The expected mortality did not occur. The remaining seven were refrigerated at 3°C. Of the twenty-three adults, twenty-two (twelve males, ten females) produced dark-veined phenotypes – again lighter than

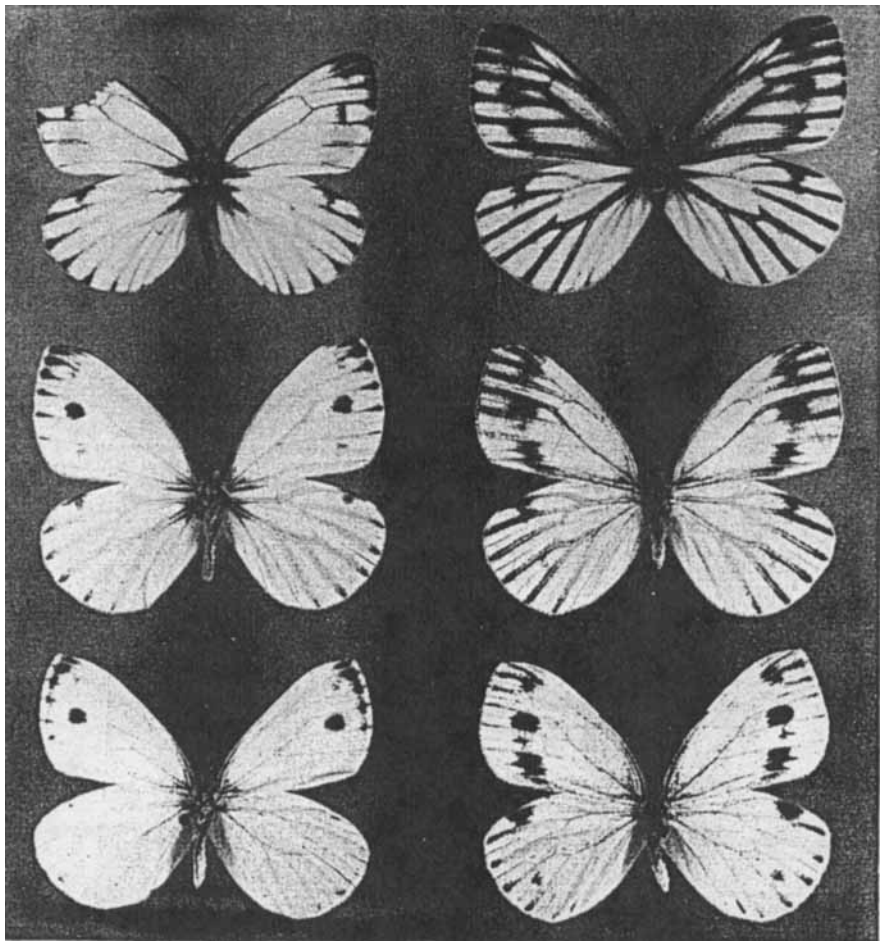

FIG. 1. Phenotypes of experimental *Pieris napi venosa* from Monterey County, California. Dorsal surfaces, males at left. Top: dark-veined phenotypes from diapaused pupae. Centre: intermediate phenotypes from chilled non-diapause pupae. Bottom: light-veined phenotypes from unchilled non-diapause pupae.

typical, dark-veined *venosa*. One female was virtually unmarked beneath. Despite the presence of one unusual female in each lot, there is no chance of an exchange.

The seventeen diapausing pupae showed no activity after 10 weeks. One developed to the pharate adult in the nineteenth week, but died without eclosing: it was a fully dark-veined male. In the thirty-second week one pupa was found to be fully pigmented and ready to eclose, and two others had laid down white but not black pigment. At this point all sixteen pupae were brought out in a dark box at 25°C, and the first adult, a male, emerged in about 3 h. Twelve more eclosed within 10 days; two showed no activity and were re-refrigerated at 3°C after a total of 14 days, and have not eclosed. Of the adults (six males, seven females) which eclosed after 32 weeks, all are very dark vernal *venosa*, as dark as their parents and darker than the veined adults from chilled non-diapause pupae. All of these phenotypes are shown in Figs. 1 and 2. The distributions of phenotypes in the chilled and unchilled non-diapause lots differ significantly ($P \ll 0.01$).

Comparisons with *P.n.microstriata*

This experiment was repeated using a fresh stock derived from two females of the interior

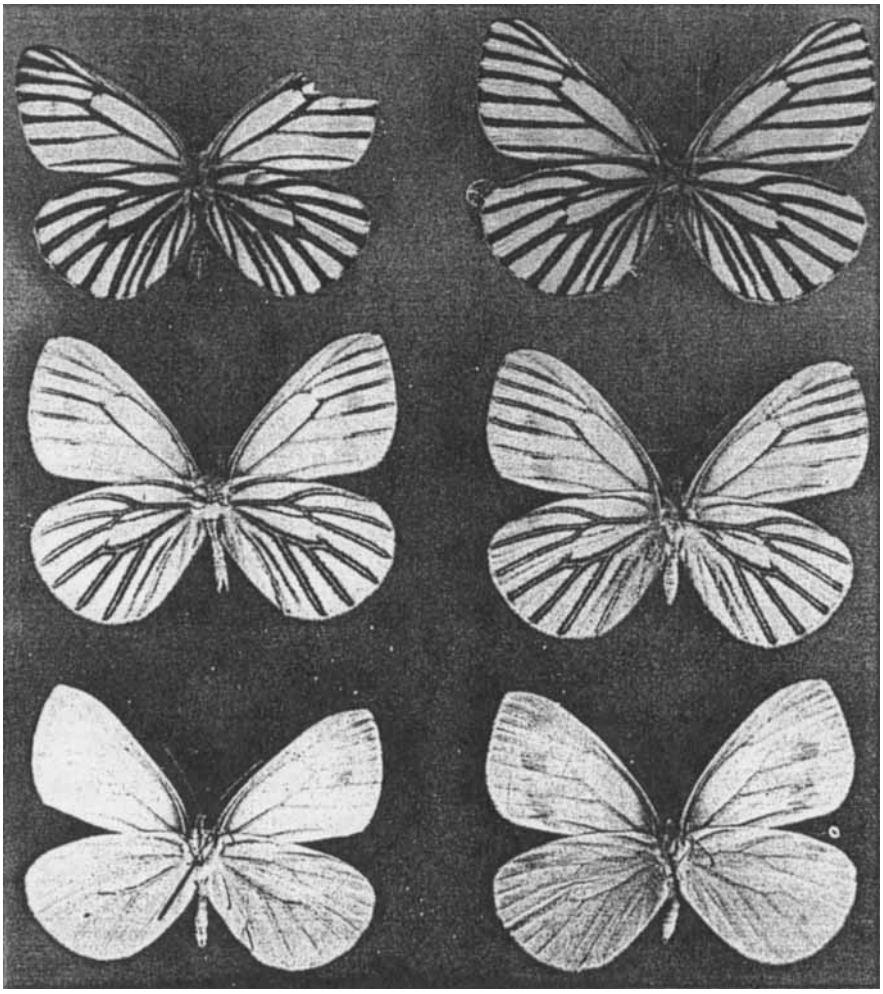

FIG. 2. As Fig. 1, but ventral surfaces.

subspecies *microstriata*, collected in Gates Canyon, Solano County, 28 March 1976 (see Shapiro, 1975, for remarks on this population). About forty ova were obtained, yielding six non-diapause, unrefrigerated adults (four males, two females), ten non-diapause, refrigerated adults (four males, six females) and fifteen diapausing pupae. All the unrefrigerated material was of the light-veined phenotype, with no dark scaling at all beneath. All but one of each sex of the refrigerated non-diapausers had some dark scales along the veins of the hindwing beneath; a few fell within the range of variation of wild spring specimens of this population. In general the effect of chilling was less dramatic in *microstriata* than in *venosa*, but its general nature and direction were the same and its lowered level of expression was consistent with the general tendency of *microstriata* to be lighter than *venosa* in all phenotypes under all regimes. Many of the diapausing pupae of this line were used in other experiments. The three animals which have eclosed (two males, one female, all after 30+ weeks at 3°C) are all normal dark-veined *microstriata* and are darker than any of the chilled non-diapause animals. The phenotypes of experimental *microstriata* are shown in Figs. 3 and 4.

Discussion and Conclusions

The present experiments demonstrate conclusively that there is an element of direct tem-

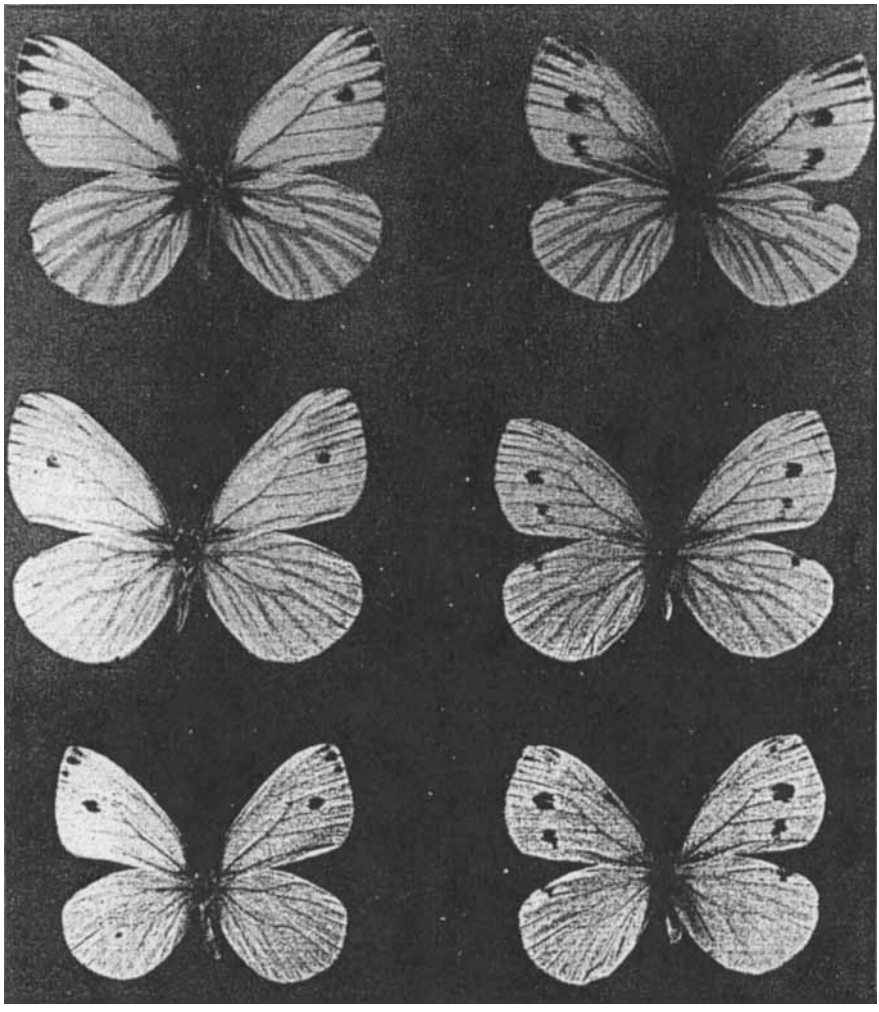

FIG. 3. Phenotypes of experimental *Pieris napi microstriata* from Solano County, California, arranged as in Fig. 1, dorsal surfaces.

perature determination of phenotype in Californian *Pieris napi*.

For the coastal, fog-belt subspecies *venosa* the ability of non-diapause pupae to give rise to dark-veined adults is almost certainly adaptive. Dark-veined *napi* fly in coastal Monterey County from January or February into April. Later individuals average somewhat lighter, and occasional dark-veined specimens can be taken into quite late spring with the light-veined phenotypes in some years. This suggests that there may be two broods of dark-veined butterflies near the coast: one of very dark animals from diapaused pupae, and a second of less dark, somewhat intermediate animals from non-diapause pupae developing in the chill of the coastal fogs. The light summer butterflies may represent relatively unchilled second-brood animals, or a partial third brood. This phenology had already been suggested to me by Mr J. Bruce Walsh of Carmel Valley, based on his collecting experience rather than on experimental data. As noted by Shapiro (1975) dark venation is probably thermoregulatorily adaptive, facilitating all activities requiring flight in the rapidly changing weather of the fog belt.

The response to chilling by *microstriata* is presumably an historical artifact, since it is univoltine and produces no non-diapause

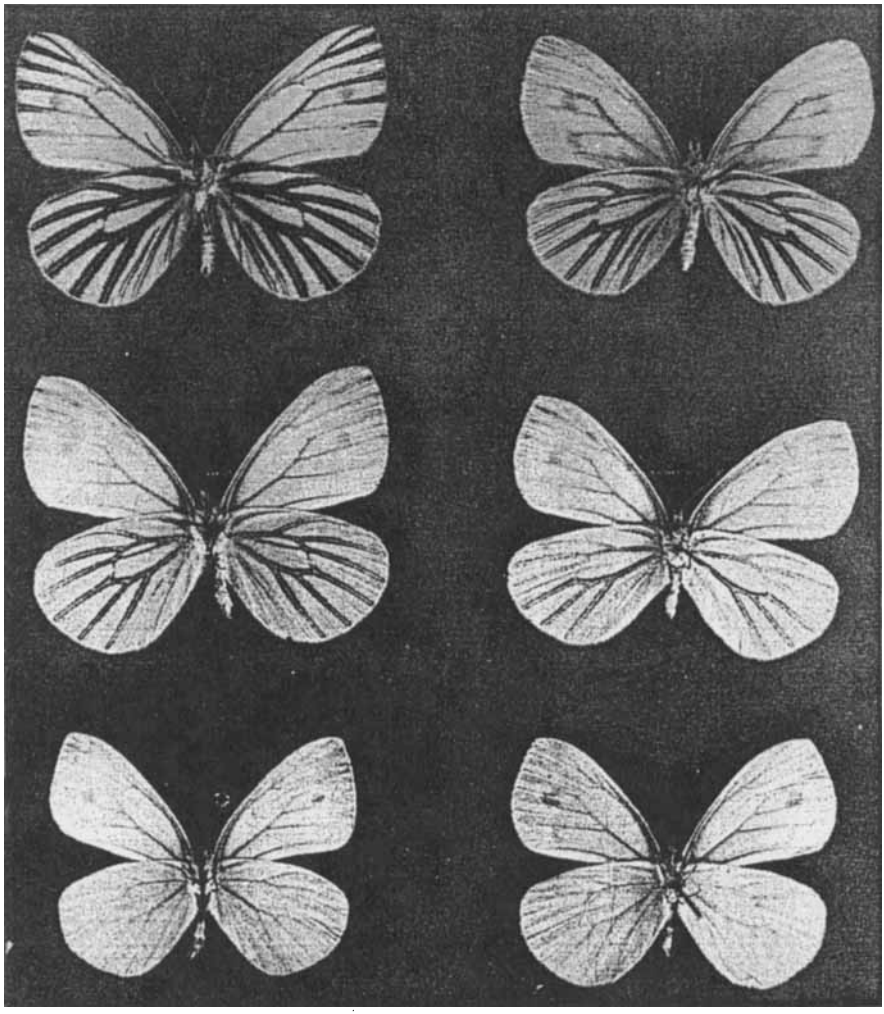

FIG. 4. As Fig. 3, but ventral surfaces.

pupae afield except in very unusual circumstances.

The most intriguing inference from these experiments is that the usual interpretation of diapause-linked polyphenism could be entirely wrong. Oliver (1970) asks whether 'it is larval photoperiod itself or the occurrence of diapause in the pupa that actually directly determines which phenotype is to be produced, since larval photoperiod and diapause cannot here be separated'. For Californian, as perhaps for European *P.napi*, it is pupal temperature and diapause which seem to require separation.

Tauber & Tauber (1976a, b) discuss the maintenance and termination of diapause and the nature of post-diapause development in insects. They state correctly (1976a) that 'the literature contains numerous stated or implied generalizations ... found to be based on little or no supportive experimental evidence derived from natural populations'. They then proceed to erect a series of 'state-of-the-art' generalizations based on their review of the field. All of these are relevant to *P.napi*, but the most important *vis-à-vis* phenotype determination reads: 'In most species with an "overwintering diapause", diapause ends by midwinter rather than in spring. Studies that are not designed to periodically test samples of populations from the field using meaning-

ful criteria of diapause termination are not suitable for establishing when diapause ends in nature.'

We have seen that *P.napi venosa* held at 3°C can develop fully to the pharate adult at that temperature, far below the larval 'developmental zero' (around 9°C), and eclose within 3 h of rewarming. If this sort of thing is usual in nature, it readily explains the commonly observed synchronized emergence of spring butterflies after only a few fine days. Suspended eclosion of pharate adults has already been identified as the cause of late autumnal 'false broods' (Shapiro, 1967, 1977). (It is unclear why reactivated diapausers proceed to the adult at temperatures as low as 3°C while non-diapausing pupae fail to develop under fairly long-term storage at 10°C.)

Diapausing *napi* pupae held at high temperatures never reactivate spontaneously, and die after 3–5 years in storage. There is some kind of a 'chilling requirement' for reactivation, though it varies considerably even among siblings. Moreover, lower temperatures than 10°C are needed to reactivate pupae within a reasonable time frame. Thus every adult which ecloses from a diapaused *napi* pupa has been chilled. Chilling while in deep diapause may have no effect on phenotype, but every adult from a diapaused pupa has presumably been chilled during the reactivation process, when pigment synthesis is going on! How, then, can one separate direct temperature effects on phenotype from those of having been in diapause? What is the basis for the consistent difference between chilled-diapause and chilled non-diapause animals? Even if one ultimately duplicates the phenotypes of diapaused *napi* precisely by chilling non-diapause pupae for long periods at temperatures near freezing, the problem is not solved. To do so one must see whether it is possible to obtain light-veined adults from diapaused pupae by manipulating the thermal environment during development. To do this, work on monitoring the respiration of diapausing pupae is continuing in the hope of 'catching' them in the initial stages of reactivation. In the meantime it is clear that there is more to seasonal phenotypic switches than was hitherto suspected.

Acknowledgments

Mr S. R. Sims and Mr J. Bruce Walsh were really responsible for this study, having contributed livestock and advice. I thank Dr John H. Crowe for invaluable discussions of diapause physiology and respirometry, and Miss P. Coutchié and Messrs J. H. Lane and S. Loomis for technical assistance. This research was sponsored in part by grant D-804 from the Committee on Research, U. C. Davis.

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The oviposition biology of siricid woodwasps in Europe

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ABSTRACT. 1. The oviposition biology of the siricid woodwasps, *Sirex noctilio*, *S.juvencus*, *S.cyaneus*, *Urocerus gigas*, *U.augur*, *U.fantoma*, *U.sah* and *Xeris spectrum* was investigated. The fecundity of each siricid species was related to adult size although *S.noctilio* laid relatively fewer eggs than other species.

2. Oviposition drill architecture in host trees is characteristic for the different siricid genera and was found to be related to the length of the ovipositor. The shape of the egg is also characteristic for each genus.

3. Species of the symbiotic fungus, *Amylostereum*, were found to be associated with all species except *X.spectrum*. The emplacement of fungus in relation to oviposition drills was investigated.

4. *S.noctilio* females have relatively larger mucus glands and reservoirs than other siricid species.

5. The response of *X.spectrum* females to previously infested timber was studied experimentally. A positive response to previously infested timber and *Amylostereum* inoculated sawdust was demonstrated.

6. The longevity of adults, oviposition rates and incubation period of eggs was investigated.

7. The results are discussed in relation to the ecology of siricids in Europe and Australia.

Introduction

The establishment of the siricid woodwasp, *Sirex noctilio* (F.), in Australasia (Miller & Clark, 1935; Gilbert & Miller, 1952; Irvine, 1962) has stimulated studies on its oviposition biology (Coutts & Dolezal, 1969; Madden, 1974) and its phytotoxic effects on trees (Coutts, 1969a, b). When *S.noctilio* attacks a host tree, it uses its ovipositor to make holes through the bark and into the wood to oviposit. During oviposition arthrospores of a symbiotic fungus, *Amylostereum areolatum* (Fries) Boidin (Gaut, 1969) are injected into the tree via the ovipositor. The arthrospores are stored in paired intersegmental sacs, the mycangia, at the base of the ovipositor (Spradbery, 1973). Fungus is introduced into the mycangia during the course of adult emergence although details of the mechanism of fungal transfer are not clear (Boros, 1968). A mucopolysaccharide secretion (King, 1966) produced by paired mucus glands and stored in a large median reservoir (Spradbery, 1973) is also injected into the wood during drilling activity. The mucus conditions living trees to fungal growth, causing rapid physiological changes in the stem and foliage (Coutts, 1969b). In combination, the mucus and fungus are frequently lethal (Coutts, 1969a, b). Of the European and North African woodwasp species, *S.noctilio* was the only one which caused phytotoxic symptoms in living trees (Spradbery, 1973).

The oviposition biology of European

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Siricidae has not been studied as intensively as *S.noctilio* in Australasia although Chrystal (1928) described the fecundity and oviposition behaviour of *Sirex cyaneus* (F.) and *Urocerus gigas* (L.) and the oviposition response of siricids to experimentally debilitated host trees was analysed by Spradbery & Kirk (1977b).

This study describes aspects of the fecundity, drilling behaviour and fungus emplacement of several European siricid species.

Materials and Methods

The siricids were obtained from infested timber collected throughout Europe, North Africa and Turkey. The timber was maintained in outdoor insectaries at Silwood Park, Ascot, and the emerging adults collected daily. For oviposition studies, females of *S.noctilio*, *S.juvencus* (L.), *Urocerus gigas*, *U.augur* (Klug.), *U.sah* Mocs. and *Xeris spectrum* (L.) were offered logs of scots pine (*Pinus sylvestris*) and sitka spruce (*Picea sitchensis*).

The females were weighed prior to dissection to provide a size index. Egg content was measured by counting the ovariole eggs which were all mature at the time of adult emergence. The mucus gland and reservoir at the base of the ovipositor were removed and weighed on glass cover-slips.

Oviposition drills, fungus emplacement and eggs laid per drill were studied by dissecting naturally infested timber and logs exposed to the woodwasps under laboratory conditions. Fungus was stained with methylene blue.

Results

Drill architecture and emplacement of fungus

The drills made in timber by ovipositing siricids were characteristic for each genus (Fig. 1). The length of the ovipositor and duration of drilling of the siricid species are given in Table 1. During drilling, siricids other than *X.spectrum* injected fungal arthrospores from the paired mycangia at the base of the ovipositor.

Sirex species made one to four drills in the wood for every insertion of the ovipositor through the bark. Single drills rarely contained eggs, the mean number of eggs per drill increasing with the numbers of drills in a group (Table 2). Drills of *Sirex* species without eggs always contained more fungus than drills with eggs. *S.cyaneus* rarely produced more than two drills per insertion but laid more eggs per single drill than the other *Sirex* species.

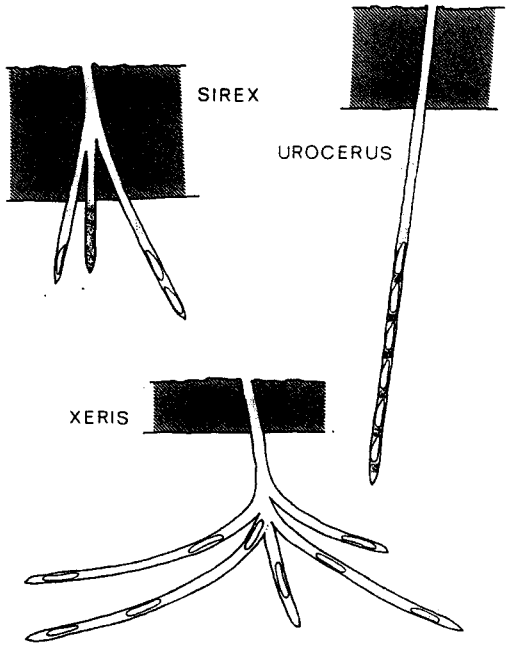

FIG. 1. Oviposition drills of *Sirex*, *Urocerus* and *Xeris* species.

The drills of *Urocerus* species are always single and relatively long and contain

TABLE 1. Length of ovipositor and duration of drilling of siricid species

Species	Length of ovipositor (mm)		Duration of drilling (min)	
	Mean	SE	Mean	SE
S.noctilio	12.4	0.401	9.4	2.383
S.cyaneus	12.7	0.237	8.7	1.324
S.juvencus	13.1	0.483	8.2	1.312
S.gigas	17.5	0.417	11.4	1.269
U.augur	20.9	1.027	18.1	1.803
U.sah	15.1	0.846	42.3	2.612
U.fantoma	13.0	0.989	15.5	1.782
X.spectrum	22.3	0.380	46.1	3.011

TABLE 2. Depth of oviposition punctures and numbers of eggs per siricid drill (data from naturally infested trees)

Species	Oviposition drills in wood*				Eggs deposited			
	Mean length (mm)	Proportion of groups			Per drill	Mean no. per:		
		Single	Double	Treble		Single	Double	Treble
S.noctilio	4.2 (0.242)	1	1.4	0.6	1.6 (0.108)	0.25	1.26	4.0
S.cyaneus	4.3 (0.241)	1	4.0	0.3	2.0 (0.135)	2.00	2.59	—
S.juvencus	4.2 (0.255)	1	0.7	0.7	1.7 (0.565)	0.77	1.83	2.3
U.gigas	9.7 (0.549)	1	—	—	4.4 (0.323)	—	—	—
U.augur	11.9 (0.829)	1	—	—	3.9 (0.376)	—	—	—
U.sah	10.6 (0.547)	1	—	—	3.1 (0.226)	—	—	—
U.fantoma	—	1	—	—	—	—	—	—
X.spectrum	12.7 (0.579)	1 (branched)			4.3 (0.512)	—	—	—

Standard errors in parentheses.
* Timber debarked before consignment.

several eggs with fungus deposited in masses between each egg (Fig. 1). *U.fantoma* has a relatively short ovipositor for its size and most naturally infested material containing this species was from trees with debarked areas. When female *U.fantoma* were offered logs with debarked patches, 76% of the drills were made at the edges of the debarked areas, 15% into the debarked wood and 9% through the bark ($n = 165$).

X.spectrum, which has a thinner and more flexible ovipositor than other siricids, made one to five branched drills in the wood with up to nine eggs per insertion.

The eggs of several siricid species are illustrated in Fig. 2. *Sirex* eggs are markedly swollen in the middle and the *Urocerus* eggs are more tapered at one end with an area of tanned protein, chorionin, at one or both ends. *Xeris* eggs are tapered at both ends with no obvious central swelling or chorionin. These generic characteristics were useful in distinguishing drills of siricids in some circumstances.

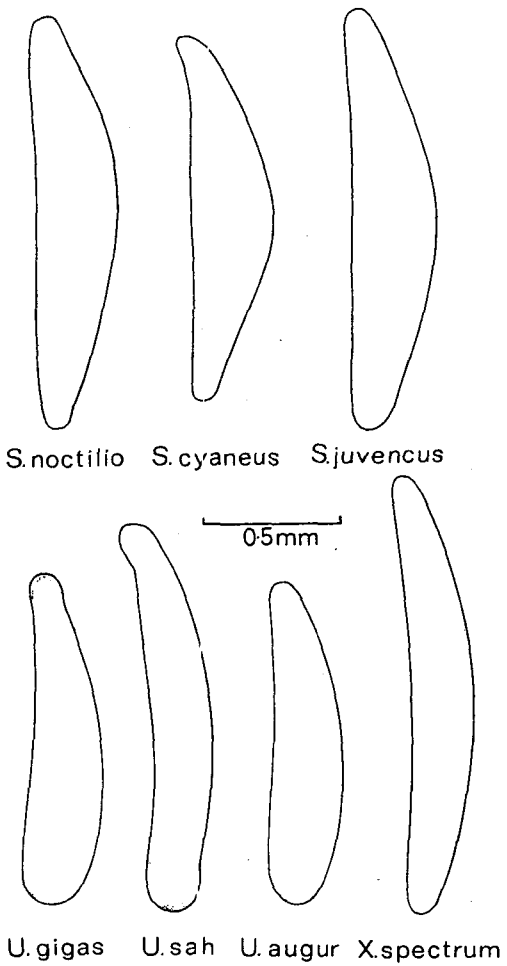

FIG. 2. Eggs of siricid woodwasps.

Fecundity and weight of mucus

The number of ovariole eggs and fresh weights of females and their mucus reservoirs are given in Table 3 and a comparison of their fecundity is given in Fig. 3. The weight of the mucus gland and reservoir in five species of siricids in relation to body weight is given in Fig. 4.

TABLE 3. Fresh weight, mucus weight and number of ovariole eggs in siricid woodwasps

Species	No. dissected	Fresh weight (mg)	Weight of mucus (mg)	No. eggs/female
S.noctilio	39	339 (82–739)	30 (10–60)	264 (136–423)
S.cyaneus	49	136 (39–389)	7 (1–23)	343 (80–858)
S.juvencus	33	147 (48–203)	9 (1–20)	299 (117–534)
U.gigas	121	318 (102–906)	14 (2–30)	647 (340–1160)
U.augur	31	218 (94–302)	13 (7–18)	515 (115–547)
U.fantoma	8	—	—	306 (102–527)
U.sah	17	411 (150–687)	23 (3–38)	465 (67–733)
X.spectrum	109	111 (36–261)	5 (1–11)	274 (42–455)

Ranges in parentheses.

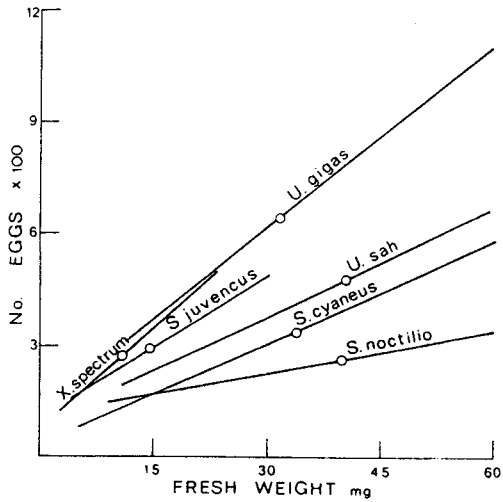

FIG. 3. Relation between fecundity and size in siricid species. For clarity, only the means are shown. Number of individuals of each species are from Table 3.

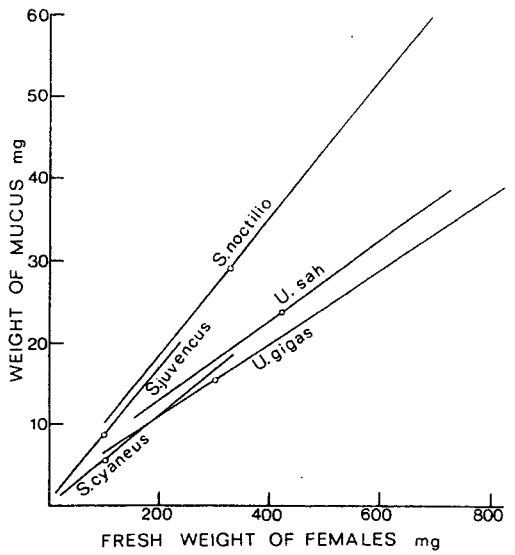

FIG. 4. Relation between weight of mucus reservoir and fresh weight of female siricids. For clarity, only the means are shown. Number of individuals of each species are from Table 3. The regression equations for each line were: *S.noctilio:* $y = 2.23 + 0.079x$, $P < 0.001$; *S.juvencus:* $y = 0.41 + 0.058x$, $P < 0.001$; *U.sah:* $y = 4.073 + 0.047x$, $P < 0.001$; *U.gigas:* $y = 1.58 + 0.048x$, $P < 0.001$; *S.syaneus:* $y = 0.46 + 0.057x$, $P < 0.001$.

Experimental study of Xeris spectrum oviposition activity

X.spectrum must rely on timber previously inoculated by other siricid species because it does not have a symbiotic fungus and its mycangia are vestigeal. On the few occasions when *X.spectrum* was observed ovipositing in the field, the chosen trees were already infested with siricids. Therefore a study was made on the response of *X.spectrum* females to currently infested, previously infested and non-infested timber. Response was determined by the number of females attracted to and ovipositing into the different logs during a 30 min observation period (twenty-five females per replicate, twenty-five replicates).

More than 85% of the females were attracted to the attacked material although there was a greater response to currently infested than to previously infested logs (Table 4). Similarly, drilling activity into currently and previously infested logs accounted for more than 96% of all drills.

The response to sawdust inoculated with *U.gigas* and *S.juvencus* symbionts was compared with the response to fungus-free sawdust controls. Samples of the sawdust were

TABLE 4. Response of *Xeris spectrum* females to siricid infested and non-infested logs

	No. females attracted	% females	No. drills	% of drills
Currently infested logs	412	51.8	341	73.4
Previously infested logs	266	33.4	108	23.2
Freshly cut logs	118	14.8	15	3.4

put in 2-cm diameter glass tubes which were fitted into holes in a sheet of polystyrene with the open ends of the tubes flush with the surface. A layer of brown paper was fitted over the surface and the apparatus introduced into a cage containing *X.spectrum* females. The number of females palpating over the tubes with their antennae and attempting to drill through the paper into the tubes was recorded during a 60 min observation period (ten replicates). There was a dominant response to *S.juvencus* inoculated sawdust compared with *U.gigas* and fungus-free controls (Table 5).

TABLE 5. Response of *Xeris spectrum* to sawdust inoculated with the symbiotic fungus of other siricids

	Mean	SE
(a) Number of palpations		
S.juvencus symbiont	36.9	2.999
U.gigas symbiont	11.6	3.732
Control sawdust	8.6	3.675
(b) Number of drills		
S.juvencus symbiont	17.0	3.330
U.gigas symbiont	5.8	4.446
Control sawdust	4.2	3.148

Longevity of adults

Some data were obtained on the longevity of adult siricids. Under laboratory conditions of 25°C and 70% R.H., *X.spectrum* females survived 7.6 days (range 2–12, $n = 109$), *S.cyaneus* 9.1 days (4–12, $n = 25$), *S.juvencus* 6.9 days (4–14, $n = 40$) and *U.gigas* 6.0 days (3–15, $n = 52$). Longevity increased to several weeks when adults were maintained in a dark room at 5°C, one *S.noctilio* female surviving for 15 weeks. By dissecting females at daily intervals it was determined that most eggs were laid during the first few days of adult life (e.g. *U.gigas* females laid 68% of their eggs within 2 days of emerging as adults).

At 25°C the eggs of *Urocerus* and *Sirex* hatched after 14 days (range 13–16) and *X.spectrum* after 12 days (range 12–13).

Discussion

The egg content of *S.noctilio* which was similar to that recorded by Madden (1974) in Tasmanian *S.noctilio,* was considerably less than that of the other siricid species. According to Chrystal (1928), *S.cyaneus* lays 300–400 eggs which agrees closely with my figures although Scheidter (1923) found an average of over 1000 eggs in *U.augur* compared to 515 (maximum 547) in my study. Because egg count is closely related to size, larger than average siricids had very high egg counts.

The oviposition drills in timber were characteristic of the different siricid genera with groups of one to four drills in *Sirex* species, single drills of greater length with several eggs in *Urocerus* species and branched drills in *Xeris*.

The pattern of oviposition by *S.noctilio* is related to the physiological condition of the host tree such that single drills without eggs indicate an exploratory or rejection response and multiple drills with eggs a favourable response (Madden, 1974). Up to five drills per group have been recorded in Australasia (Morgan & Stewart, 1966). The mean number of eggs per single drill in naturally infested trees was 0.04 in Tasmania (Madden, 1974) compared to 0.25 in my study. The difference may well be due to the greater incidence of attack on living trees in Australasia (Rawlings & Wilson, 1949) compared to Europe (Spradbery & Kirk, 1977a) for more single drills are made in the initial stages of attack when the physiological condition of the host tree is 'normal' (Madden, 1974). Because the number of eggs per drill probably indicates the host tree's suitability (Spradbery & Kirk, 1977b), dead or moribund trees would be expected to stimulate more egg-laying in single drills compared with living trees.

Urocerus injected arthrospores between successive eggs as they were laid in the drill, in contrast to the *Sirex* species which injected fungus into the final, egg-less drill of a group (Coutts & Dolezal, 1969). *S.cyaneus* drills were somewhat intermediate in style with several eggs laid in series within the drill (Table 2) and the fungus not always confined to an egg-less drill in a group. Chrystal (1928) recorded one to three eggs per single *S.cyaneus* drill and did not remark on any differences from *U.gigas* drills.

The positive response of *X.spectrum* females to timber previously inoculated with the symbiotic fungus of other siricid species was confirmed experimentally. The low response to the *U.gigas* symbiont, *Amylostereum chailletii* (Pers. ex Fr.) Boidin, in comparison with that of the *S.juvencus* symbiont, *A.areolatum*, may be due to differential growth rates and subsequent metabolite production by the two fungus species. However, there was only one locality from which *X.spectrum* was recorded which produced *U.gigas* as an exclusive associate and eleven other localities which produced *Sirex* species as exclusive associates. All other *X.spectrum* localities had both genera present (Spradbery & Kirk, 1977a). These results suggest that *X.spectrum* may be specifically attracted by the *A.areolatum* symbiont.

The fact that the mucus reservoir of *S.noctilio* is significantly larger than that of other siricids has an important ecological implication. It was established that *S.noctilio* is the only European species of siricid which produces a phytotoxic mucus (Spradbery, 1973) and that this mucus in combination with the symbiotic fungus is lethal to living trees (Coutts, 1969b). Because *S.noctilio* is outstanding in synthesizing a toxic mucus and does so copiously probably explains why this species has become an important pest of Australian pine forests. By contrast, *X.spectrum* which has no symbiotic fungus (Stillwell, 1966) and oviposits only in timber previously infested by other siricid species has the smallest mucus reservoir.

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