

**An analysis of geographical variation in the parasitoid *Rhyssa persuasoria* (L.) (Hymenoptera, Ichneumonidae)**

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**Abstract**

Studies were made of populations of adult *Rhyssa persuasoria* (L.) from various geographical regions. Morphometric analyses were based on the relative size of 32 maculae on various sclerites and the colour of the coxae and legs. Size was determined by measuring the wing length and thorax length. Various statistical procedures were applied to these data and also the ratios of wing length : maculation, and thorax length : maculation in an attempt to distinguish races of *R. persuasoria* from different localities. Regression analysis, analysis of variance and classification procedures separated some populations from others, the results of the different analyses being in broad agreement with each other. Within each population, maculation was typically linear, smaller specimens having relatively small maculae. Some sexually dimorphic differences were also demonstrated. There were two major geographical groupings: S.W. European and North African (W. France, Spain, Portugal and Morocco) and the remainder. There were some geographical differences within the remaining populations although all had links with one or more adjacent populations. The degree of maculation was shown to be related to the colour and texture of host-tree species, populations with low maculation scores being associated with tree species having dark, fissured bark and *vice versa*. It is suggested that degree of maculation is an expression of cryptic colouration, providing camouflage from predators. Determinations of heat-coma temperature for male *R. persuasoria* from climatically different areas showed that the response to high temperature differed between populations and was correlated with the temperature of the locality of origin. These differences between strains were apparent after several months of identical conditioning and are probably genetically based.

**Introduction**

In any sample of an insect species, variation in structure, colour or physiology may be found, these differences being due to genetic polymorphism and/or the effects of a variable environment (Richards, 1961). When the variation is genetically based, the species may utilise this capacity to obtain better adjustment to its environment.

An opportunity to study variation in the Ichneumonid parasitoid of Siricid woodwasps, *Rhyssa persuasoria* (L.), was provided by the surveys initiated by CSIRO for

natural enemies of Siricids in Europe, Turkey and North Africa. *R. persuasoria* occurs throughout much of the Holarctic region where it occupies localities with different climatic and ecological conditions. A morphological feature of *R. persuasoria* adults is the size and number of pale spots or maculae which range in colour from white to yellow on the otherwise black-pigmented sclerites. Maculae are frequently used by taxonomists to separate insects at the species level (Townes & Townes, 1960). During routine determinations of insect material emerging from Siricid-infested timber, it was noted that *R. persuasoria* from some countries had large maculae on most sclerites, whereas from other countries the maculae were small in size and few in number (F. Wilson, pers. comm.). A study was made to determine the range of variation in relation to the geographical distribution of the parasitoid and attempt to relate phenetic differences to environmental factors. Because there is evidence that natural selection operates on a suite of characters rather than on individual characters (Blackith & Reymont, 1971), much of the mathematical enquiry was applied to the sums or means of the morphometric parameters.

As a corollary to the morphometric study, the separation of physiologically different races of *R. persuasoria* was attempted, using a heat-coma bioassay technique. Using a similar method but based on chill-coma, Free & Spencer-Booth (1961) distinguished the European honeybee, *Apis mellifera* L., from the Indian species, *A. indica* F., by their responses to temperature.

Extreme colour forms, i.e., those exhibiting large and many maculae and those with small and few maculae, were crossed and compared with their progeny to provide some information on the genetic basis of the variation.

#### Materials and methods

The insects used in this study were reared from Siricid-infested timber from Europe, Turkey and North Africa. Timber was collected by one of the authors (J. P. S.) and a CSIRO officer, A. A. Kirk, during the period 1963-70 and stored in outdoor insectaries at Ascot, England, according to locality of origin and host tree species. All dead and dying conifers were sampled for Siricid larvae and their parasitoid complex, and any material found infested was collected. The low incidence of Siricid-infested trees in the countries visited provided no opportunities for selecting material and virtually all infested material found was shipped to England. It is considered that timber thus collected represents random samples from the areas visited. Emerging adults were collected and identified and every fifth specimen of each species from each locality was pinned and stored. The number of emerging parasitoids and hosts was extremely variable, depending on the original infestation level, degree of parasitism and the time of collection. The duration of the immature stages of Siricids is from one to three years, that of the parasitoids occupying only one year. As a result, material yielded Siricids and parasites together or separately. Where possible, 25 specimens of each sex were preserved. However, the demands of the biological control programme for female parasitoids and a sex ratio of 9 : 1 in favour of males resulted in few females of *R. persuasoria* being available for this study. The populations of *R. persuasoria* used in the morphometric study represent most localities for which preserved material was available. The localities are given in Table I together with the numbers of sites, trees and logs available.

To determine the degree of maculation, the relative area occupied by the maculae on various sclerites was scored 0-5 according to size: 0, absent; 1, very small; 2, small; 3, medium; 4, large; 5, very large or coalescing with other maculae. The positions of maculae used in the study were as follows (Fig. 1a,b):

- |                |                  |                          |
|----------------|------------------|--------------------------|
| 1. temple      | 5. upper notum I | 9. upper mesepisternum   |
| 2. inner orbit | 6. lower notum I | 10. median mesepisternum |
| 3. clypeus     | 7. coxa I        | 11. lower mesepisternum  |
| 4. gena        | 8. tegula        | 12. mesoscutellum        |

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Fig. 1-

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|----------------------|-----------------------|------------------------|
| 13. notum III        | 20. lower tergite III | 27. upper tergite VI   |
| 14. mesepimeron      | 21. upper tergite III | 28. lower tergite VII  |
| 15. coxa II          | 22. lower tergite IV  | 29. upper tergite VII  |
| 16. propodeum        | 23. upper tergite IV  | 30. lower tergite VIII |
| 17. metapleuron      | 24. lower tergite V   | 31. upper tergite VIII |
| 18. lower tergite II | 25. upper tergite V   | 32. tergite IX         |
| 19. upper tergite II | 26. lower tergite VI  |                        |

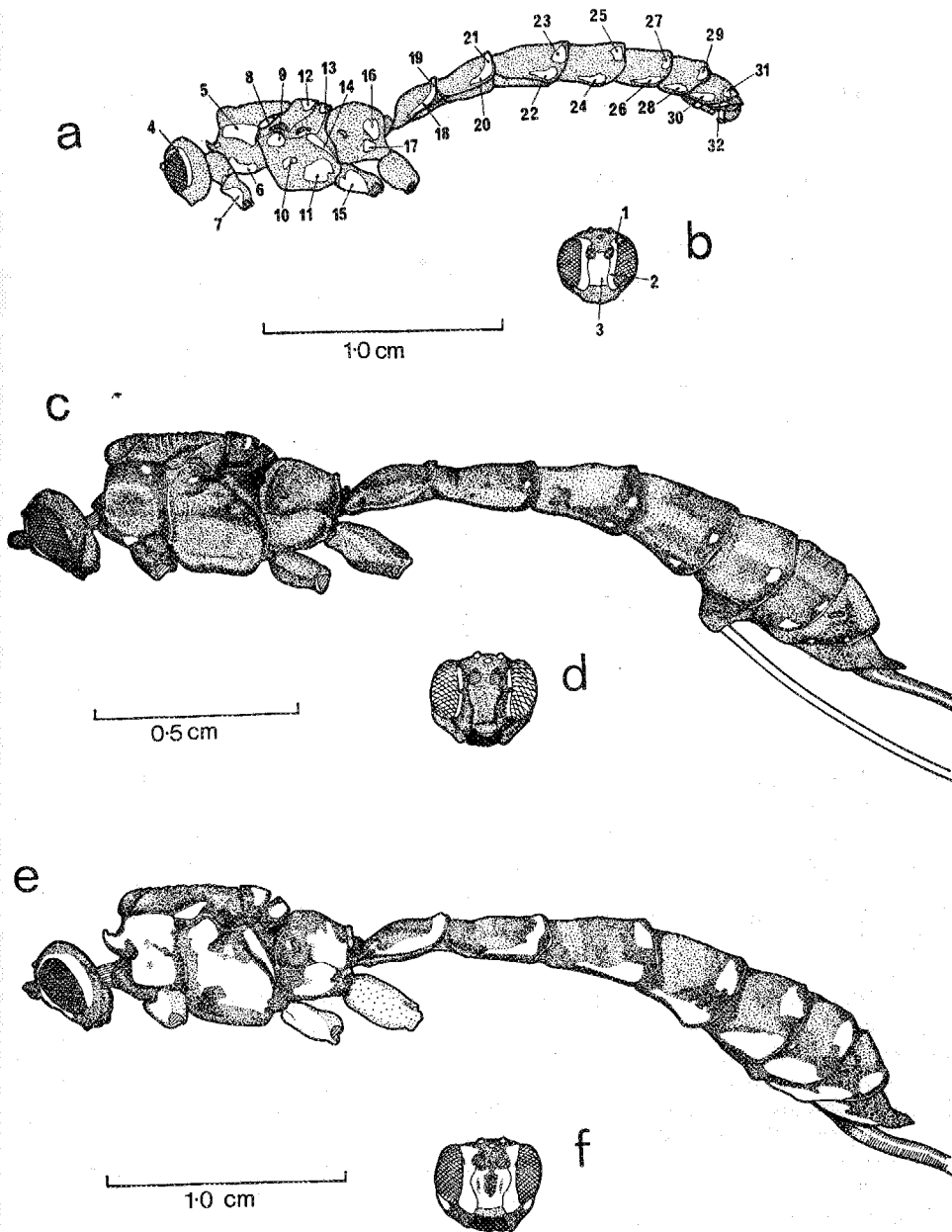


Fig. 1.—Maculation of *Rhyssa persuasoria*. a, b, maculae used in morphometric study; c, d, female from Morocco; e, f, female from Ireland.

upper mesepisternum  
 median mesepisternum  
 lower mesepisternum  
 mesoscutellum

In a separate morphological analysis, the colour of the coxae was scored 1-5 according to their colour: 1, all pale yellow; 2, some brown and some yellow; 3, all brown; 4, dark brown, with occasionally one pair, usually coxae III, black; 5, all black (Fig. 2). A further parameter, the colour of the legs (Fig. 2) was assessed by scoring each segment of each leg (coxa, trochanter, femur, tibia and tarsus) 0-5 according to colour: 0, all black; 1, dark brown; 2, mid brown; 3, light brown; 4, brown or black with yellow patches; 5, predominantly yellow. The size of the specimens was determined by measuring the

TABLE I. Localities and number of sites, trees and logs for collections of *Rhyssa persuasoria*

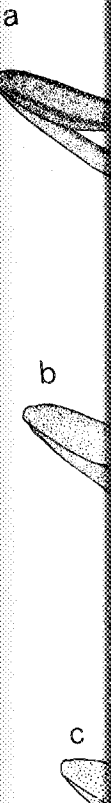
No.	Locality	No. collecting sites	No. trees collected	No. logs collected
1	England 1	1	3	26
2	England 2	2	15	53
3	N. Germany	2	5	53
4	C. Germany	1	2	20
5	N. France	3*	6	27
6	S.E. France	2	13	69
7	Norway	2	2	43
8	Sweden	2	5	19
9	Italy 1	4	18	64
10	Italy 2	1	4	53
11	Turkey 1	1	1	6
12	Turkey 2	4	6	36
13	Czechoslovakia	1	1	26
14	Switzerland	5	27	132
15	Ireland	5	11	38
16	Bulgaria	1	1	13
17	Scotland	1	1	22
18	Holland	1	1	5
19	Yugoslavia	4	18	60
20	Greece	3	4	20
21	W. France	1	2	12
22	N.W. Spain	5	39	142
23	Portugal	2	56	317
24	Morocco	3	10	35

\* The three sites in N. France were geographically very close.

length of the forewing from tegula to wing tip and the length of thorax and propodeum from the anterodorsal edge of notum I to the petiole, using a micrometer eyepiece. A quantitative measure of the darkness of tree bark from which *R. persuasoria* emerged was obtained by scoring for bark colour 1-5: 1, light; 2, medium light; 3, medium; 4, medium dark; 5, dark; presence of dark fissures 0-3: 0, absent; 1, few; 2, frequent; 3, abundant; and pale coloured bark scales 0-2: 0, absent; 1, frequent; 2, abundant. The bark scale score was subtracted from bark colour plus fissure scores to derive bark rating.

To determine physiological differences, adult males were subjected to high temperature to determine their heat-coma temperature. Shortly after emergence, single insects were placed in tubes with honey and water and acclimatised in the dark at 20.0°C for 24 h. They were then transferred to 2.5-cm-diameter glass tubes containing water 1 cm deep to ensure approximately 100% r.h. Above the water level was a 2-cm compartment formed by two discs of polystyrene in which the insect was confined. The bulb of a thermometer (accuracy  $\pm 0.01^\circ\text{C}$ ) traversing the cork, reached the centre of the compartment. The tubes were placed in an electrically stirred water bath at 25°C for one hour before the temperature of the water was slowly increased at a constant rate of 0.15°C per minute. The temperature was noted when the insects became comatose as indicated

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xae was scored 1-5 according to the yellow; 3, all brown; 4, black; 5, all black (Fig. 2). A key scoring each segment of leg to colour: 0, all black; 1, black with yellow patches; 2, determined by measuring the

by a cessation of normal body movements and doubling of the legs beneath the body. Uncoordinated twitching of the legs and abdomen frequently accompanied this generally well defined response. Insects later found by dissection to contain nematodes were rejected and the forewing of the remainder was measured as an index of body size.

Dark and light forms of *R. persuasoria* were crossed after collecting virgin females from Spanish and Irish material, obtaining mating with the opposite colour form and rearing their progeny from Siricid-infested logs. The statistical and classificatory procedures used to analyse geographical variation are described in the results.

for collections of

Days	No. logs collected
26	53
53	20
27	69
43	19
64	64
53	6
36	26
132	132
38	13
22	5
60	20
12	142
317	35

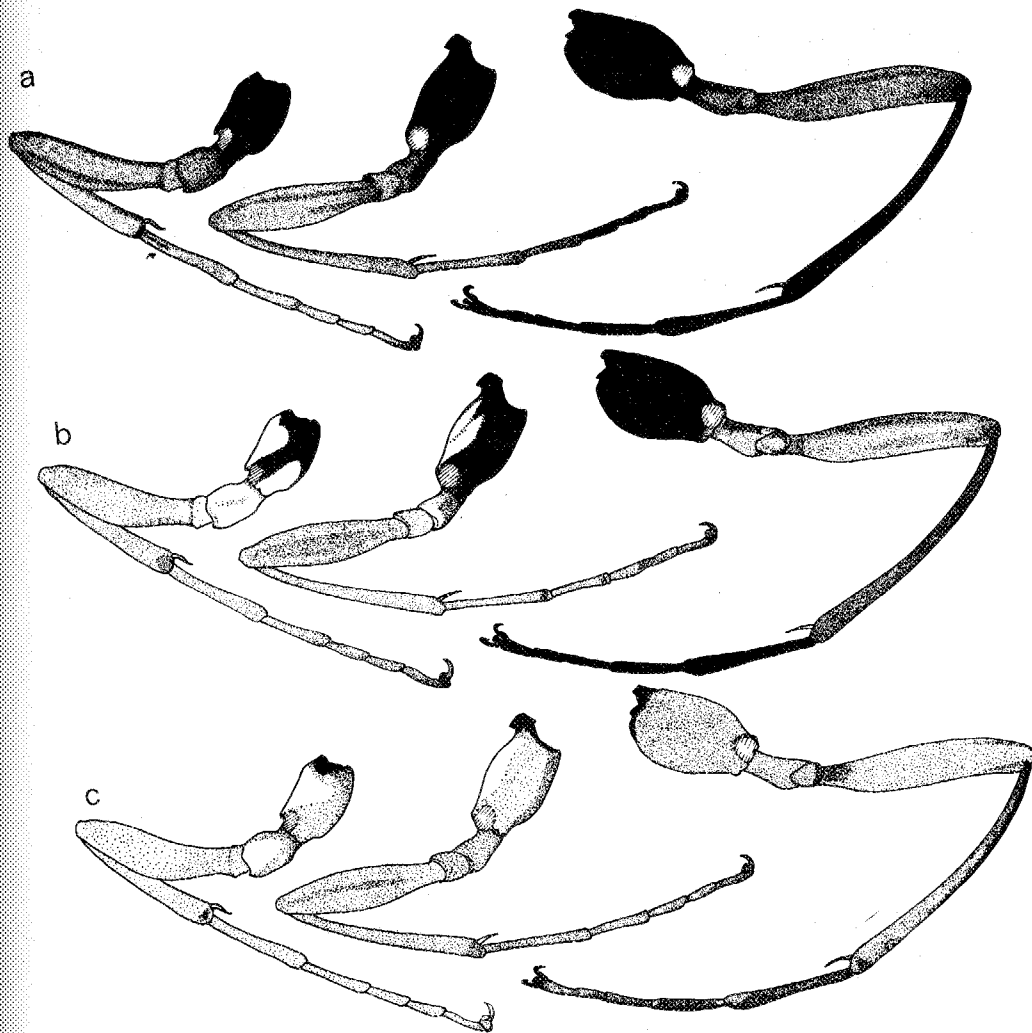


Fig. 2.—Legs of *Rhyssa persuasoria* males. a, Moroccan; b, Swedish; c, Irish.

of thorax and propodeum with a micrometer eyepiece. A *R. persuasoria* emerged was scored as light; 3, medium; 4, dark; 1, few; 2, frequent; 3, abundant. The scores to derive bark

subjected to high temperature emergence, single insects were kept in the dark at 20.0°C for 24 hours containing water 1 cm deep in a 2-cm compartment. The bulb of a test tube was placed at the centre of the compartment at 25°C for one hour at a constant rate of 0.15°C per hour. The insects were comatose as indicated

## Results

### Morphological study

In samples of *R. persuasoria* from different countries there was considerable variation in the maculation scores of individuals and this was directly related to size. Large specimens tended to have more and larger maculae and small specimens fewer and smaller maculae. Differences between males and females were also demonstrated. Although females were generally larger than males and had correspondingly higher maculation scores, there were also other dimorphic differences, maculum 3 being almost or completely absent and maculum 2 much reduced in females, while in males these maculae were all fully developed except in very small specimens. A paired sample *t*-test on the means of the ratio, maculation : thorax length, showed that males and females were significantly different ( $P < 0.01$ ).

Other occasional features of maculation were the dorsal coalescence of the propodeal maculae (16), lateral coalescence of the upper and lower maculae on tergite II (18 and 19) and tergite III (20 and 21) and dorsal coalescence of the maculae on tergite II (19).

A summary of morphometric and maculation data is given in Table II. In this summary and subsequent analyses, the sums of the individual scores for the 32 body maculae, and the legs and the mean of the coxae scores were used because it was considered a more significant description of the overall appearance of the insect, the phenotype on which selection must work. The range of maculation score (the sum of the scores for the 32 individual maculae) for each population was due to differences in the size of the specimens. Scatter diagrams of maculation (*y*) on thorax length (*x*) indicated a linear relationship between the two parameters (Fig. 3). Significant differences were also

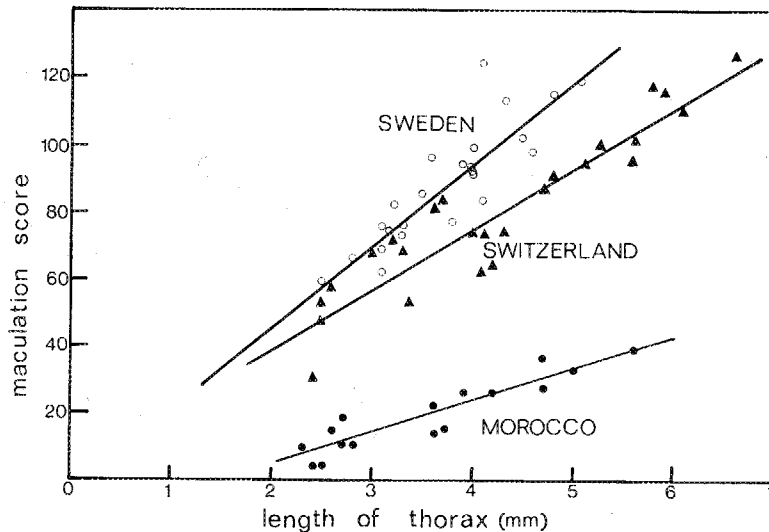


Fig. 3.—Relationship between maculation score and length of thorax for three samples of *R. persuasoria*.

demonstrated in the slopes of the regression lines between some populations (extreme examples are shown in Fig. 3). To overcome within-population differences due to size, and because the intercepts of most of the regression lines of *y* on *x* were sufficiently close to zero, the ratios of maculation : thorax length ( $M/TL$ ) and maculation : wing length

( $M/WL$ ) were calculated for Norway and Portugal, Spain and

Preliminary Turkish and maculation treatment first series analysis revealed (i.e., Norway and Spain) Norway and Although *R. persuasoria* consistency lower, individuals. There were links with

A further sub-population have been therefore. These demonstrated from Devon major areas north-east of the Tamar, Cornwall, distinguished demonstrated though both. Significant originating respectively.

Data homogeneous different varieties did differ. Swiss material.

The regression procedure disregarded agglomerations in which because exhibited strategies the single of cluster elements. mathematical. Since similar form that is the correlation that between

( $M/WL$ ) were determined. The most discrete populations were a Scandinavian group (Norway and Sweden) with a high maculation ratio and a south-western group (Morocco, Portugal, Spain and W. France) with a low ratio.

Preliminary statistical analyses were made in an attempt to classify the European, Turkish and North African males using a one-way analysis of variance on each of the two maculation ratios and on the morphometric variables. Pairs of significantly different treatment means were separated by the method of least significant differences. In the first series of tests which combined the two English and two German localities, the analysis revealed a few obvious differences, notably the distinctness of the Scandinavian (*i.e.*, Norway and Sweden) and south-western groups (*i.e.*, Portugal, Morocco, W. France and Spain) from the remainder. The mean  $M/TL$  and  $M/WL$  ratios for insects from Norway and Sweden were significantly higher than those of all other populations. Although their means were indistinguishable, the estimated variance of Swedish *R. persuasoria* was much lower than that of Norwegian specimens, indicating less internal consistency in the latter. The maculation ratio of the south-western group was significantly lower than that of all other populations and their variances were also much lower, indicating greater internal consistency in the maculation of darker specimens. There were also some subgroupings within the remaining populations, but all subgroups had links with adjacent populations and there were no clear-cut geographical separations.

A further feature which emerged from this analysis was the apparent existence of sub-populations within a population, indicating that specimens from one country may have been collected from two or more different localities. Analyses of variance were therefore made on the  $M/TL$  ratio for four countries exhibiting such heterogeneity. These demonstrated no differences between the two English populations of *R. persuasoria* from Devon/Dorset and Norfolk. In the Irish material, which was derived from three major areas, significant differences ( $P < 0.01$ ) were demonstrated between insects from the north-east (Glenarm, Co. Antrim and Newcastle, Co. Down) and the south-west (Kenmare, Co. Kerry) although a northerly group (Barons Court, Co. Tyrone) was not distinguished from either of these populations. Slight differences ( $P < 0.05$ ) were demonstrated between the two groups comprising the northern France population although both were collected within a few kilometres of each other in the Alençon district. Significant differences ( $P < 0.01$ ) were demonstrated between Yugoslavian populations originating from Croatia and Montenegro, their mean  $M/TL$  being 23.783 and 17.825, respectively.

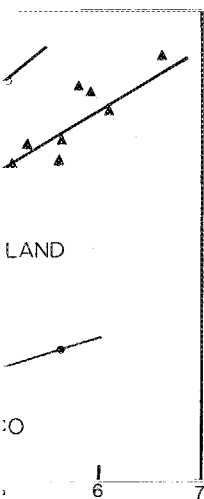
Data for Sweden, Ireland, Switzerland and Morocco were compared to test the homogeneity of their variance. Irish and Swiss *R. persuasoria* were not significantly different with respect to their individual variance, but Swedish and Moroccan populations did differ significantly ( $P < 0.05$ ) although they had a smaller variance than the Irish and Swiss material.

The next stage of the statistical analysis considered classification or cluster analysis procedures based on the mean value of each character for each of the groups and thus disregarded information on individuals. The classification methods chosen were agglomerative hierarchical and the indices of dissimilarity used were Euclidean distance in which each character was scaled to unit variance. The Euclidean distance was chosen because each of the three characters used, *viz.*  $M/TL$ , coxae rating and legs rating, exhibited more-or-less continuous variation over its range. Several different sorting strategies were employed, all of which are discussed by Cormack (1971). These included the single linkage (nearest neighbour) strategy in which the similarity between an entity of cluster and that of another is defined as the measure or distance between their closest elements. This strategy was shown by Jardine & Sibson (1968) to satisfy a set of mathematical criteria to which they consider any classificatory strategy should conform. Since single linkage tends to incorporate an entity into existing clusters rather than have it form the core of an independent cluster, it is weakly clustering. At the other extreme is the complete linkage (furthest neighbour) strategy in which the similarity is defined as that between the most remote pair of elements, one in each group. This strategy is

There was considerable variation directly related to size. Large small specimens fewer and s were also demonstrated. had correspondingly higher es, maculum 3 being almost males, while in males these ens. A paired sample *t*-test ved that males and females

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some populations (extreme ion differences due to size, on  $x$  were sufficiently close i maculation : wing length

TABLE II. *Morphometric data of Rhyssa persuasoria*

No.	Geographical origin	No. specimens	Sex	Wing length (mm)	Thorax length (mm)	Maculation score (0-160)	Coxae rating (1-5)	Legs rating (0-75)	$M/\bar{W}L$	$M/\bar{W}L$	Tree species
1	England 1 (Norfolk)	16	♂	10.4 (7.1-13.4)	4.1 (3.1-5.6)	56.9 (35-106)	2.83 (2.0-3.5)	29.6 (27.0-39.5)	5.30 (1.9-8.7)	13.94 (8.3-22.9)	<i>Pinus sylvestris</i>
2	England 2 & 3 (Devon, Dorset)	20	♂	14.5 (8.0-23.3)	5.4 (3.1-8.5)	83.8 (32-134)	2.33 (1.5-3.5)	—	6.02 (3.1-8.5)	15.98 (8.2-22.4)	<i>Larix decidua</i>
3	N. Germany (Reinbeck)	9	♂	10.0 (7.4-13.0)	3.9 (3.1-4.7)	75.3 (56-91)	2.44 (2.0-3.5)	29.5 (26.0-31.0)	7.57 (6.67-8.6)	19.29 (17.1-22.9)	<i>P. sylvestris</i>
4	C. Germany (H-Münden)	21	♂	11.4 (6.7-14.7)	4.4 (2.5-5.7)	91.1 (49-115)	1.98 (1.5-3.0)	29.8 (26.0-31.0)	8.03 (6.3-10.3)	20.85 (16.1-27.1)	<i>P. sylvestris</i>
		11	♀	14.5 (10.4-19.6)	5.3 (3.9-6.4)	97.0 (83-114)	—	—	6.81 (6-8.3)	18.70 (15.6-21.9)	"
5	N. France (Alençon)	18	♂	10.6 (6.5-14.2)	4.1 (2.5-5.3)	71.3 (45-108)	2.19 (1.5-3.0)	29.8 (26.5-34.5)	6.94 (3.3-9.0)	18.09 (8.7-22.8)	<i>Abies alba</i>
6	S.E. France (Turini)	25	♂	11.2 (5.5-17.5)	4.2 (2.2-6.2)	80.8 (35-114)	2.34 (1.5-3.5)	31.8 (30.0-33.5)	7.46 (3.2-9.2)	19.52 (7.8-25.3)	<i>A. alba</i>
		20	♀	15.4 (11.2-24.4)	5.7 (4.1-8.7)	96.8 (52-136)	—	—	6.31 (5-8)	17.2 (13.3-21.4)	<i>P. abies</i>
7	Norway (Oslo)	25	♂	8.3 (5.2-12.8)	3.2 (2.2-5.0)	74.8 (47-104)	1.98 (1.5-2.5)	35.5 (35.5-35.5)	9.05 (6.9-11.2)	23.19 (12.1-27.2)	"
		4	♀	10.9 (10.5-11.8)	4.0 (3.7-4.2)	75.5 (62-85)	—	—	6.93 (5.8-7.9)	18.92 (14.8-21.1)	"
8	Sweden (Stockholm)	25	♂	9.9 (6.8-13.0)	3.8 (2.5-5.1)	88.3 (59-124)	2.02 (2.0-2.5)	35.7 (35.5-37.5)	8.96 (7.5-10.2)	23.54 (20-26.7)	"
		5	♀	14.1 (13.3-15.0)	5.1 (4.9-5.7)	106.6 (99-114)	—	—	7.56 (7.4-7.7)	20.74 (20.0-21.1)	"
9	Italy 1 (Arezzo)	15	♂	10.2 (6.5-14.0)	3.9 (2.5-5.1)	81.8 (54-112)	2.20 (1.5-3.0)	—	8.07 (6.7-9.6)	20.98 (17.4-24.6)	<i>A. alba</i>
		7	♀	16.2 (14.5-18.9)	5.7 (5.2-6.7)	119.1 (114-126)	—	—	7.37 (6.6-8.3)	20.99 (18.7-23.8)	"
10	Italy 2 (Sabaundia)	7	♂	13.8 (11.8-15.2)	5.2 (4.8-6.0)	110.2 (100-140)	1.79 (1.5-2.0)	28.3 (26.5-30.0)	8.06 (6.6-9.3)	21.11 (17.3-23.3)	<i>Pinus radiata</i>
		8	♀	16.4 (11.5-19.2)	6.1 (4.6-7.1)	123.1 (103-139)	—	—	7.64 (6.9-9.0)	20.36 (18.4-22.5)	"
11	Turkey 1 (Kazdaz)	15	♂	8.3 (6.3-10.7)	3.4 (2.7-4.3)	54.9 (35-72)	2.73 (2.5-3.0)	20.0 (20.0-20.0)	6.60 (4.5-9.0)	16.36 (10.9-21.1)	<i>Pinus brutia</i>
12	Turkey 2 (Trabzon)	25	♂	10.1 (6.2-15.8)	3.7 (2.6-6.0)	74.7 (42-111)	2.08 (1.5-2.5)	29.9 (24.5-33.5)	7.43 (6.0-10.0)	20.02 (7.6-25.9)	<i>Picea orientalis</i>
		12	♀	15.1 (11.3-22.7)	5.4 (4.1-8.1)	102.2 (79-127)	—	—	6.88 (5.6-8.1)	19.48 (17.2-23.2)	"
13	Czechoslovakia (Banska Stavnica)	25	♂	9.4 (6.4-11.7)	3.5 (2.3-4.3)	69.2 (51-98)	2.02 (1.5-2.5)	24.7 (18.5-28.0)	7.42 (5.5-9.7)	19.70 (14.6-26.3)	<i>A. alba</i>
14	Switzerland (Délemont)	25	♂	10.8 (6.6-18.3)	4.2 (2.4-6.6)	79.5 (31-126)	1.70 (1.5-2.0)	34.1 (27.0-39.0)	7.25 (5.3-8.8)	18.58 (12.1-22.5)	"
		16	♀	14.6 (12.2-19.7)	5.3 (3.9-6.7)	100.4 (54-138)	—	—	6.93 (4.4-8.2)	18.76 (12.3-22.0)	<i>P. abies</i>
									17.85 (14.6-26.3)	17.85 (14.6-26.3)	<i>L. decidua</i>



10	Italy 2 (Sabaunia)	7	♀	16.2 (14.5-18.9)	5.7 (5.2-6.7)	119.1 (114-126)	—	—	7.37 (6.6-8.3)	20.99 (18.7-23.8)	"
		7	♂	13.8 (11.8-15.2)	5.2 (4.8-6.0)	110.2 (100-140)	1.79 (1.5-2.0)	28.3 (26.5-30.0)	8.06 (6.6-9.3)	21.11 (17.3-23.3)	<i>Pinus radiata</i>
		8	♀	16.4 (11.5-19.2)	6.1 (4.6-7.1)	123.1 (103-139)	—	—	7.64 (6.9-9.0)	20.36 (18.4-22.5)	"
		15	♂	8.3 (6.3-10.7)	3.4 (2.7-4.3)	54.9 (35-72)	2.73 (2.5-3.0)	20.0 (20.0-20.0)	6.60 (4.5-9.0)	16.36 (10.9-21.1)	<i>Pinus brutia</i>
		25	♂	10.1 (6.2-15.8)	3.7 (2.6-6.0)	74.7 (42-111)	2.08 (1.5-2.5)	29.9 (24.5-33.5)	7.43 (6.0-10.0)	20.02 (7.6-25.9)	<i>Picea orientalis</i>
		12	♀	15.1 (11.3-22.7)	5.4 (4.1-8.1)	102.2 (79-127)	—	—	6.88 (5.6-8.1)	19.48 (17.2-23.2)	"
		25	♂	9.4 (6.4-11.7)	3.5 (2.3-4.3)	69.2 (51-98)	2.02 (1.5-2.5)	24.7 (18.5-28.0)	7.42 (5.5-9.7)	19.70 (14.6-26.3)	<i>A. alba</i>
14	Switzerland (Délemont)	25	♂	10.8 (6.6-18.3)	4.2 (2.4-6.6)	79.5 (31-126)	1.70 (1.5-2.0)	34.1 (27.0-39.0)	7.25 (5.3-8.8)	18.58 (12.1-22.5)	"
		16	♀	14.6 (12.2-19.7)	5.3 (3.9-6.7)	100.4 (54-138)	—	—	6.93 (4.4-8.2)	18.76 (12.3-22.0)	<i>P. abies</i>
		25	♂	13.0 (7.1-21.9)	5.1 (2.7-8.5)	87.6 (41-132)	1.87 (1.0-2.5)	36.0 (29.0-38.0)	6.94 (3.5-8.8)	17.85 (9.3-23.8)	<i>L. decidua</i> <i>Abies nobilis</i>
		20	♀	17.17 (13.7-23.5)	6.5 (5.0-8.8)	107.5 (59-143)	—	—	6.06 (4.1-7.0)	17.0 (11.8-20.7)	<i>Picea sitchensis</i>
		4	♂	14.1 (13.1-15.6)	5.3 (5.0-5.8)	90.5 (76-98)	2.13 (2.0-2.5)	25.4 (23.0-32.5)	6.43 (5.8-7.0)	17.05 (15.2-18.2)	<i>A. alba</i>
		20	♂	11.1 (6.7-15.3)	4.2 (2.1-5.6)	68.8 (33-99)	2.45 (1.5-4.0)	35.7 (32.0-36.0)	6.23 (3.4-8.8)	16.44 (8.9-19.5)	<i>L. decidua</i>
		6	♀	15.0 (12.5-16.2)	5.4 (4.5-6.1)	79.0 (53-111)	—	—	4.95 (3.4-6.9)	14.65 (9.5-19.6)	"
		10	♂	11.1 (8.0-15.0)	4.3 (3.2-5.9)	90.3 (55-123)	1.80 (1.0-2.5)	35.6 (31.0-37.5)	8.02 (6.6-10.1)	20.93 (16.7-28.7)	<i>P. abies</i>
		8	♀	14.1 (11.1-17.1)	5.2 (4.1-6.3)	87.3 (28-113)	—	—	6.1 (2.5-7.1)	16.63 (6.8-19.8)	"
		10	♂	10.7 (8.1-15.4)	4.1 (3.0-5.8)	84.9 (61-108)	2.20 (1.5-3.0)	27.5 (24.0-30.0)	8.02 (5.8-10.6)	21.40 (16.3-27.8)	<i>A. alba</i>
		9	♀	16.7 (12.2-23.6)	6.2 (4.4-8.9)	106.1 (79-141)	—	—	6.38 (6.0-6.8)	17.39 (15.8-18.9)	<i>P. abies</i>
		5	♂	10.8 (9.4-12.8)	4.1 (3.6-4.7)	84.8 (66-114)	1.80 (1.5-2.0)	26.0 (26.0-26.0)	7.86 (6.4-10.7)	20.74 (17.4-27.8)	<i>Abies cilicica</i>
		9	♂	11.5 (7.2-14.6)	4.6 (2.9-5.8)	30.2 (15-45)	3.67 (3.0-5.0)	22.1 (18.5-22.5)	2.60 (1.5-3.4)	6.51 (3.6-8.0)	<i>Pinus pinaster</i>
		15	♂	10.9 (7.2-17.7)	4.2 (2.8-6.5)	39.1 (23-66)	3.97 (3.5-4.5)	12.1 (12.0-13.5)	3.36 (2.2-7.4)	8.43 (5.2-20)	<i>P. pinaster</i> <i>P. radiata</i>
		3	♂	9.2 (7.6-10.0)	3.6 (3.0-4.1)	19.0 (16-24)	4.17 (4.0-4.5)	16.5 (16.5-16.5)	2.07 (1.6-2.4)	5.30 (3.9-6.3)	<i>P. pinaster</i> <i>P. radiata</i>
		15	♂	9.4 (5.6-15.0)	3.6 (2.3-5.6)	20.8 (5-39)	4.65 (3.0-5.0)	13.0 (13.0-13.0)	2.15 (0.8-3.3)	5.45 (2-7.9)	<i>Pinus halepensis</i>

Ranges in parentheses

intensely clustering and tends to form a large number of clusters. Both single linkage and complete linkage require only an ordering of the indices of similarity and hence it was possible to carry out the present calculations without a computer. The third strategy employed was group average, which leads to a clustering intensity somewhat intermediate between single and complete linkage.

TABLE III. Results of classification of *R. persuasoria* using single-linkage and complete-linkage sorting strategies

Populations and characters employed in classification	Groups obtained using	
	Single linkage	Complete linkage
1-24 <i>M/TL</i> , Coxae and Legs	(a) 21-24 (b) 1-20	(a) 7-8, 14-15, 18 (b) 21-24 (c) 1-3, 5-6, 17 (d) 4, 9-10, 12-13, 16, 19-20 (e) 11
1-20 <i>M/TL</i> , Coxae and Legs	(a) 7-8, 14-15, 18 (b) 2-6, 9-10, 12-13, 16, 19-20 (c) 1 (d) 11 (e) 17	(a) 7-8, 14-15, 18 (b) 2-3, 5-6, 17 (c) 4, 9-10, 12-13, 16, 19-20 (d) 1, 11
1-20 <i>M/TL</i> and Coxae	(a) 7-8 (b) 14-15 (c) 3-6, 9-10, 12-13, 16, 18-20 (d) 2, 11, 17 (e) 1	(a) 7-8 (b) 14-15 (c) 2-3, 5-6, 16-17 (d) 4, 9-10, 12-13, 18-20 (e) 1, 11

Results of the cluster analyses are shown in Table III and Fig. 4. With data from all localities and for all three characters, the single linkage strategy gives rise to only two groupings, one of these being the south-western group previously obtained and composed exclusively of the darker specimens from S.W. Europe and Morocco. The complete linkage strategy not only confirms the south-western group, but indicates a subgrouping consisting of the northerly populations from Norway, Sweden, Holland, Ireland and Switzerland. A further subgrouping encompasses the remaining populations from southern and north-western Europe. The final large subgrouping consists of those populations which lie to the south and east of the Alps, exceptions being central Germany which is included in this group and the Turkish (Kazdaz) population which is not in this or any other group.

Because the four south-western populations (nos. 21-24) had *M/TL*, coxae and legs rating scores quite different from that of the remaining populations, a further analysis was carried out using only localities 1-20. These data more nearly approximate a normal distribution than obtained when all 24 localities are considered. The results for the complete linkage analysis are essentially similar to those previously obtained. The results for single linkage indicate a tendency, although there are exceptions, to unite all the populations from continental Europe. The third analysis shown in Table III uses localities 1-20 and only two characters, viz., *M/TL* and coxae rating. The results are substantially the same as before except that the specimens from Holland are no longer associated with the Scandinavian populations but are found grouped with more southerly populations. This results from the fact that whereas the legs rating for Holland was very high and of the same order of magnitude as for Norway and Sweden, the mean *M/TL* and coxae scores are rather more comparable with continental populations. This serves to illustrate the importance of choice of variable in cluster analysis.

In Fig. 4 are shown the results of the classification using the group-average strategy. The heights of the line junctions above the horizontal correspond to increasing levels of dissimilarity. The inferences are much the same as those derived from Table III, but

Fig. 4 serves and further groupings in the far reliability a

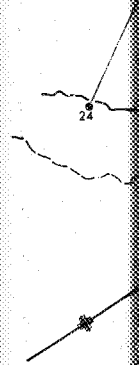


Fig. 4.—G

sampling more clear one site at and Holla The d principal

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s of similarity and hence it  
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ustering intensity somewhat

Fig. 4 serves to pinpoint more exactly the locations from which collections were made, and further illustrates the general tendency towards the existence of continental subgroupings on either side of the Alpine chain, and towards additional insular subgroupings in the far northerly populations. Reference to Table I shows that differing degrees of reliability are to be attached to the various collections because of differing degrees of

- using single-linkage  
ies
- ed using
- Complete linkage
- (a) 7-8, 14-15, 18
  - (b) 21-24
  - (c) 1-3, 5-6, 17
  - (d) 4, 9-10, 12-13, 16, 19-20
  - (e) 11
- (a) 7-8, 14-15, 18
  - (b) 2-3, 5-6, 17
  - (c) 4, 9-10, 12-13, 16, 19-20
  - (d) 1, 11
- (a) 7-8
  - (b) 14-15
  - (c) 2-3, 5-6, 16-17
  - (d) 4, 9-10, 12-13, 18-20
  - (e) 1, 11

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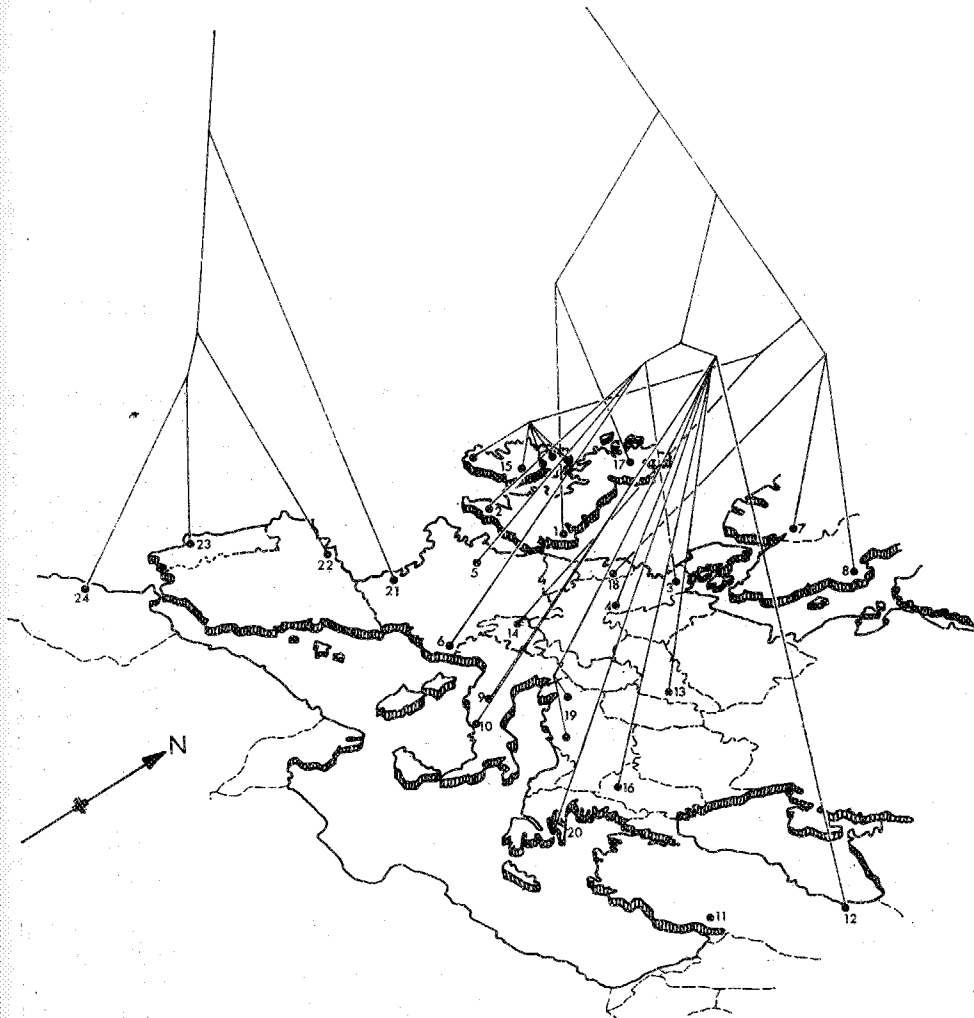


Fig. 4.—Graphic representation of results of hierarchical classification using the group-average strategy (numbers from Table II).

sampling intensity. Some of the population samples tending to form a bridge between more clearly defined subgroupings are seen to be those that have been collected at only one site and derived from only one or two trees, as in central Germany, Turkey (Kazdaz) and Holland.

The data for *R. persuasoria* males listed in Table II seemed suitable for use in a principal coordinates analysis (Gower, 1967). The results are shown in Fig. 5, the three

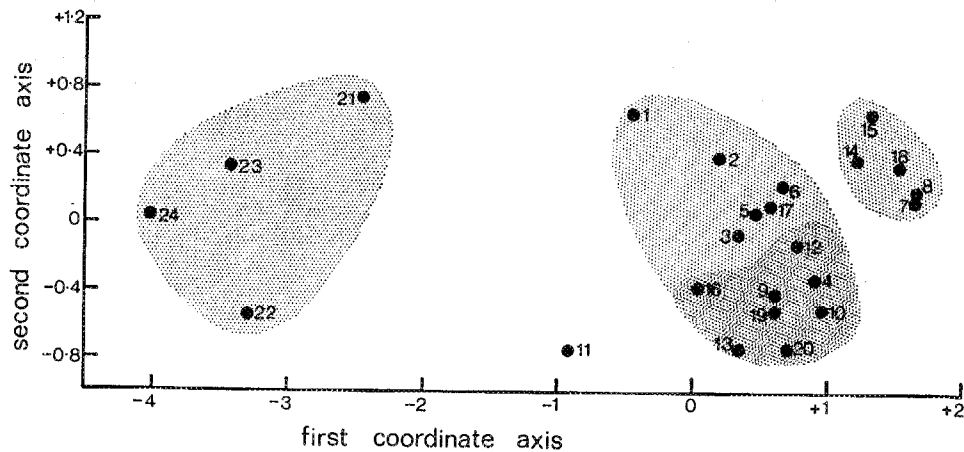


Fig. 5.—Principal coordinate analysis of *Rhyssa persuasoria* (numbers from Table II).

characters  $M/TL$ , coxa rating and leg rating having been used. The first coordinate axis score, and the second co-ordinate axis is positively correlated with leg rating. Thus darker samples appear on the left of the Figure, lighter ones on the right. The south-western population (nos. 21–24) forms a distinct group widely separated from the remainder. The Turkey 1 population is widely separated from the remainder. There is a northerly group on the right of the Figure and an assemblage of populations loosely defined as a central European group with low leg rating and a more northerly group comprising material from England 1 and 2, Scotland, N. and S.E. France. The coloration of the legs would appear to be the more important parameter separating the populations. The coordinates analysis clearly supports the results of hierarchical classification and there appears to be general agreement between these methods of analysis.

To determine the effects of environment on the colour pattern of *R. persuasoria*, three factors were considered; the number of trees and localities from which each sample was derived, climatic factors and the host-tree species with its characteristic bark colour and texture. Some samples were derived from single trees and others from several trees distributed over a wide area and it was considered that the large variance within a sample might result from widely dispersed collection sources. However, a comparison between variance of  $M/TL$  and degree of source dispersion (number of collection points, plus 1, 2 or 3 to denote one, few or several tree sources) showed no significant correlation ( $r = 0.135$ , 23 d.f.,  $P > 0.10$ ).

There was no significant relationship between  $M/TL$  and a number of geographical and meteorological parameters (mean annual temperature, precipitation and altitude).

The relationship between  $M/TL$  and the darkness of host tree bark is presented in Fig. 6 and shows a reasonable correlation between these two parameters such that 'darker' populations (*i.e.*, low maculation scores) were associated with trees having darker and more heavily fissured bark. The only notable exception was the population from Italy 2, Sabaudia.

#### Physiological study

Heat-coma data for adult male *R. persuasoria* from several climatically different localities are summarised in Table IV. There was an increase in mean temperature of heat-coma from central European or high altitude localities to coastal Mediterranean areas. There were significant correlations between heat-coma temperature and the mean temperature for June (the principal flight period) and the mean annual temperature of

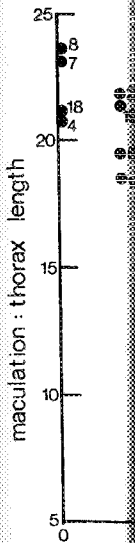


Fig. 6.—

the locality individual

#### Cross-mating

To determine the effect of cross-mating on the canonical discriminant function analysis of a population, a series of experiments were conducted. The greater the distance between the individuals from same locality, the greater the additional of experimental error.

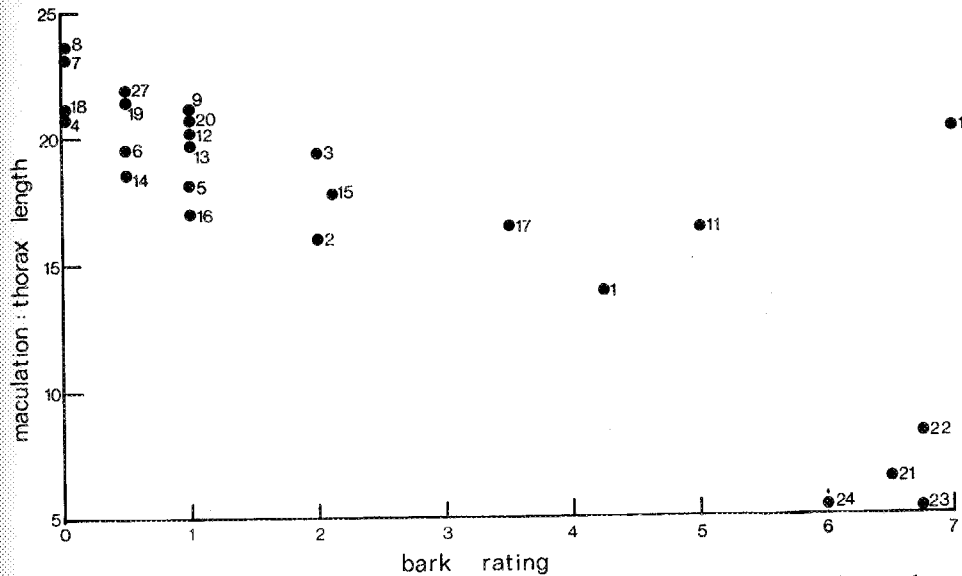
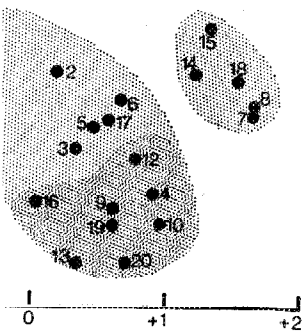


Fig. 6.—Relationship between the ratio, maculation score : thorax length, and bark colour rating of host tree species.

TABLE IV. Heat-coma temperatures of *Rhyssa persuasoria* males

Geographical origin	No. used	Heat-coma temperature (°C)	
		Mean	± s.e.
S.E. France	13	38.78	0.46
Switzerland (Bulle)	24	39.31	0.14
Czechoslovakia	38	39.47	0.17
Yugoslavia (Crotia)	3	40.23	0.23
Italy (Arezzo)	6	40.45	0.28
N.W. Spain	16	40.90	0.23
Greece	5	41.50	0.21
Turkey (Kazdaz)	24	41.89	0.32
Corsica	25	41.90	0.11

the locality of origin (Fig. 7). Within the populations there were no correlations between individual heat-coma and body size.

*Cross-mating studies*

To determine relationships between parental populations and their progeny from the cross-mating experiments, the *M/TL*, coxa score and leg rating parameters were analysed canonically. The discriminant analysis makes use of the variation between the individuals of a population such that the more similar the individual values of a population, the greater the distinctness of populations. The analysis was made with the computer program *BMDO7M Stepwise Discriminant Analysis* (Dixon, 1970). The closeness of the individual progeny to a parental sample was assessed by the Mahalanobis squared distance. The analysis computes a set of linear discriminant functions based on data from samples of known origin. The discriminant functions are used then to classify additional material of unknown origin into one of the original populations. The results of experiments using Irish and Spanish populations are given in Fig. 8. To facilitate ease of reading, the individual members of the parental samples are not shown in Fig. 8,

(numbers from Table II).

1. The first coordinate axis is related with leg rating. Thus points on the right are widely separated from the other populations. The southern assemblage of populations is more northerly and a more northerly N. and S.E. France. The parameter separating the results of hierarchical classification methods of analysis. The southern of *R. persuasoria*, three characteristic bark colour and others from several trees. The variance within a sample, a comparison between of collection points, plus 1, no significant correlation

a number of geographical precipitation and altitude). bark is presented in two parameters such that associated with trees having perception was the population

several climatically different in mean temperature of to coastal Mediterranean temperature and the mean an annual temperature of

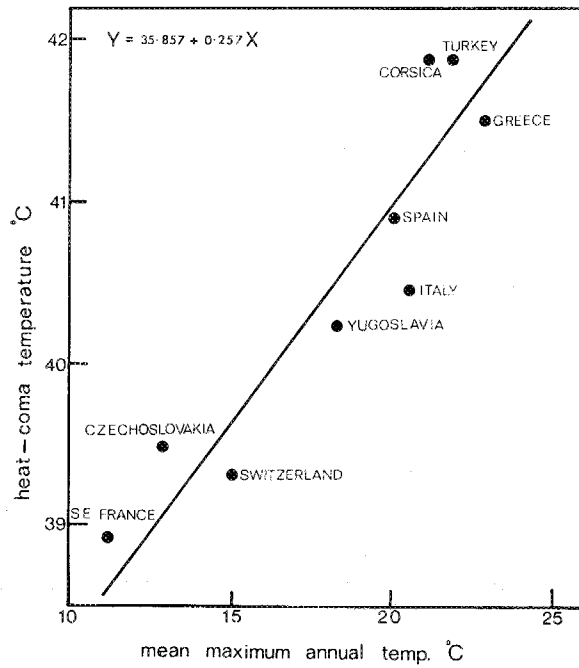


Fig. 7.—Relationship between mean heat-coma temperature of male *Rhyssa persuasoria* samples and the mean annual temperature of the locality of their origin.

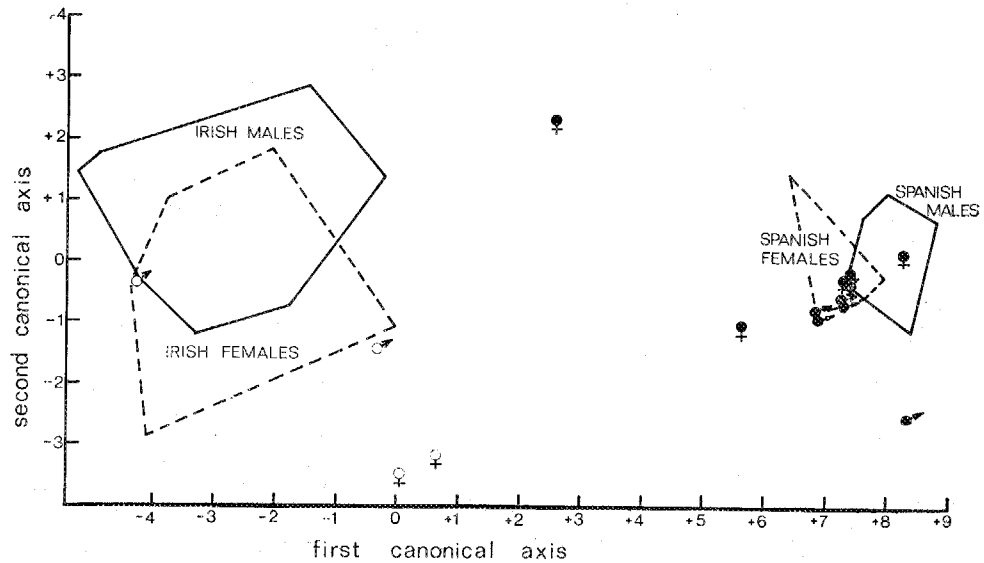


Fig. 8.—Discriminant analysis of Spanish and Irish parents and their progeny. (Polygons delimit parental coordinates; open circles, progeny of Irish females; closed circles, progeny of Spanish females.)

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but their extent is indicated by the polygons. Although the numbers of female progeny were few, their positions in Fig. 8 illustrate a range of  $F_1$  progeny from those which were indistinguishable from the female parent to intermediate forms. This figure also illustrates the greater internal variation in the Irish population of *R. persuasoria* compared with the darker Spanish population.

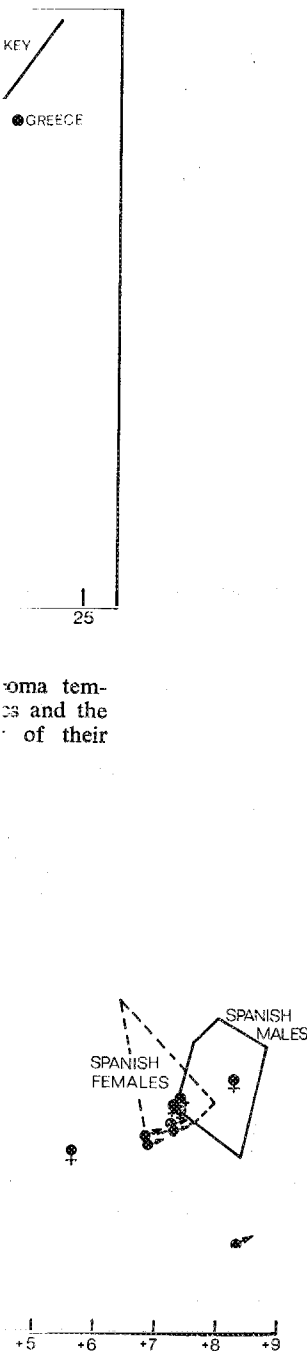
### Discussion

Considerable variation is concealed in 'normal' individuals and populations which consist chiefly of recessive gene alleles and gene complexes such that no two individuals in a sexually outbreeding species are likely to be genetically identical (Dobzhansky, 1972). Difference between individuals may vary according to their size and sex while these and broader inter-population differences can be marked or subtle. Polymorphic forms or ecotypes may be identified by morphological and/or physiological criteria and attempts can be made to relate these differences of characteristics of their habitat.

In populations of *R. persuasoria*, several broad geographical categories were morphometrically displayed and classified. Some of the variation between individuals was due to size and was typically linear, while sexual dimorphism accounted for some minor differences. Among European, N. African and Turkish populations, discontinuous variation was demonstrated between a south-western group and the remaining populations. Within the last category, subgroups were demonstrated although variation was continuous and each subgroup had links with one or more adjacent populations. The geographical isolation of Norway and Sweden and the isolation of the south-western group imposed by the distribution of coastal pine and the Pyrenean mountain barrier have probably contributed to the development and maintenance of these differences. In continental Europe, there is virtually complete continuity of conifers and Siricid hosts and a uniformity of host tree genera. With the lack of barriers and probably age-old inter-mixing of insect populations, augmented by modern commercial movements of timber, the development or persistence of distinct geographical varieties is less likely. However, with the exception of one population (central Germany), the Alpine range appears to be the formation of two subgroups, a northern subgroup and a south eastern subgroup.

Differences between populations showed no correlation with climatic factors but a relationship between colour forms and the dominant host tree species was established. The darker-looking populations, those with relatively small maculae, were associated with species of *Pinus* which have characteristically dark and heavily fissured bark. Conversely, lighter-looking populations were found in regions where *Picea* and *Abies* predominated, these two tree genera having light-coloured bark with pale scales and few, if any, dark fissures. Although the assessment of bark colour is arbitrary, such an association between the insect and its host tree appears to have an adaptive basis. It seems likely that adult *R. persuasoria*, which spend a considerable time on the trunks of infested trees (males awaiting emerging females and females ovipositing in the wood) would be vulnerable to predators hunting selectively by sight. There would therefore be selective advantage in some form of cryptic colouration to afford camouflage against the background of the tree. Cryptic colouration can be a transient polymorphism brought about by such factors as predator pressure and can be considered extremely labile. There can be several solutions to a problem of camouflage in an insect species, broadly determined by its genotype and influenced by environmental factors and the often subtle differences between some of the subgroups of *R. persuasoria* can probably be accounted for in this way.

Physiologically different populations of *R. persuasoria* were demonstrated by heat-coma determinations. That these differences are genetically controlled appears certain in view of the identical conditions of acclimatisation of the different strains for several months before experiments were made. Differences in response to temperature were



their progeny. (Polygons represent males; closed circles, progeny of

not correlated with maculation, which therefore must have diversified in response to other factors.

Although some female progeny of the cross-mating experiments were intermediate in colour, four of the six bred from dark Spanish females were identical with the female parents and none of the progeny closely approached the paternal coordinates. Male progeny, which are haploid (produced from unfertilised eggs), closely resembled the female parent from which they derive all their genetic material.

#### Acknowledgements

We wish to thank the National Pinetum, Bedgbury, England, for supplying valuable information on trees. Dr W. T. Williams provided a much appreciated stimulus during the early stages of the taximetric analysis. Funds were provided to one of us (J.P.S.) by the National Sirex Committee.

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