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A TEST OF THE MECHANICAL ISOLATION HYPOTHESIS IN TWO SIMILAR SPIDER SPECIES

Amy D. Ware

Route 2, Box 1964
Bumpass, Virginia 23024 USA

and

Brent D. Opell[1]

Department of Biology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061 USA

ABSTRACT

External reproductive organs of spiders are often species-specific and are important taxonomic characters in species identification. One explanation of this is the mechanical isolation or lock-and-key hypothesis. It predicts that in closely related species with overlapping ranges, morphological character displacement should occur in regions of sympatry to prevent costly interspecific copulation attempts. To test this hypothesis, we measured homologous external genital sclerites of male *Larinioides (Nuctenea) sclopetaria* and *L. patagiata* (Araneidae) and statistically compared their means and variances for sympatric and allopatric regions of their distribution. Differences in both mean and variance were observed, but the number of sclerites that differed between regions of sympatry and allopatry was not greater than the number that differed between adjacent regions of sympatry. Thus, these species failed to demonstrate the character displacement predicted by the mechanical isolation hypothesis.

INTRODUCTION

In spiders, as in many invertebrate groups, genitalic differences have been widely used to distinguish species. The "lock-and-key" mechanism of reproductive isolation has traditionally been used to explain these differences in the sclerotized, external genitalia of both male and female spiders. It postulates that the genitalia are specifically shaped so that during mating they couple correctly only between conspecifics (Mayr 1963). Although this hypothesis is commonly used to explain the often small genitalic differences which separate species, it has never been put to a critical test. Grasshoff's (1973a, b, 1975) descriptions of the mechanisms that couple male and female genitalia make it plausible that small differences in one or several genitalic parts (sclerites) could preclude mating between related species. However, for most species the precise functions of genitalic sclerites are unknown and the amount of difference sufficient to prevent successful mating is unclear. Additionally, only Coyle (1985) has quantified the genitalic variation in a

[1] Address reprint requests to B. Opell.

population of spiders and no study has analyzed the pattern of variation over a species' range.

Eberhard (1985) raises several objections to the traditional lock-and-key or mechanical exclusion mechanism, the first being that it was originally proposed for insects in which the female genitalia are relatively rigid, whereas the genitalia in many other groups are not rigid and, therefore, probably incapable of mechanical exclusion. Another objection is that species-specific sclerites of the male genitalia of some species contact parts of the female genitalia that do not differ among species, a situation inconsistent with the lock-and-key explanation. A strong theoretical objection to the mechanical isolation hypothesis is that selection should favor females that can identify a potential mate's species early in courtship (prior to physical contact), since courtship and copulation are costly for a female (Daly 1978).

As alternatives to the lock-and-key mechanism, Eberhard (1985) suggests four hypotheses to explain the often species-specific nature of animal genitalia: (1) species isolation by genitalic stimuli, (2) pleiotropic effects of alleles selected in other contexts, (3) mechanical "conflict of interest" between males and females, and (4) sexual selection by female choice. Eberhard's evidence more strongly supports the latter hypothesis.

The objective of this study is to evaluate the mechanical isolation hypothesis for species-specific genitalic differences by testing two of its predictions. If, as this hypothesis predicts, genitalic differences prevent costly and potentially dangerous interspecific copulation attempts between closely related species, then genitalic features of partially sympatric species should show greater differences in regions of sympatry than in regions of allopatry. Additionally, if two species with similar genitalia are influenced by interspecific competition for mates, their genitalia should be less variable within areas of sympatry than within areas of allopatry. To test these predictions we measured and compared homologous genitalic structures of two similar spider species.

MATERIALS AND METHODS

Criteria for selecting study species.—Before selecting study species, we established four requirements. First, the species' taxonomy must have been recently revised to insure that they are valid morphological species and to provide a current picture of their distribution. Second, the species must have similar genitalic structures and occur in areas of sympatry and allopatry only with one another, or both their ranges must be completely sympatric with one or more closely related species. This is necessary to insure that any detected effects do not result from interaction between a third species and only one of the two study species. Third, species must have sufficient size and seasonal overlap to make interbreeding a real possibility. Fourth, an ample number of museum specimens must be available for study.

Surprisingly few spider species meet all of these requirements; however, two species of Araneidae, *Larinioides sclopetaria* (Clerck) and *Larinioides patagiata* (Clerck), qualify, the systematics of their genus having been recently revised by Levi (1974). Subsequently, Grasshoff (1983) transferred these and two other species from *Nuctenea* to *Larinioides* Caporiacco 1934. Although both species are

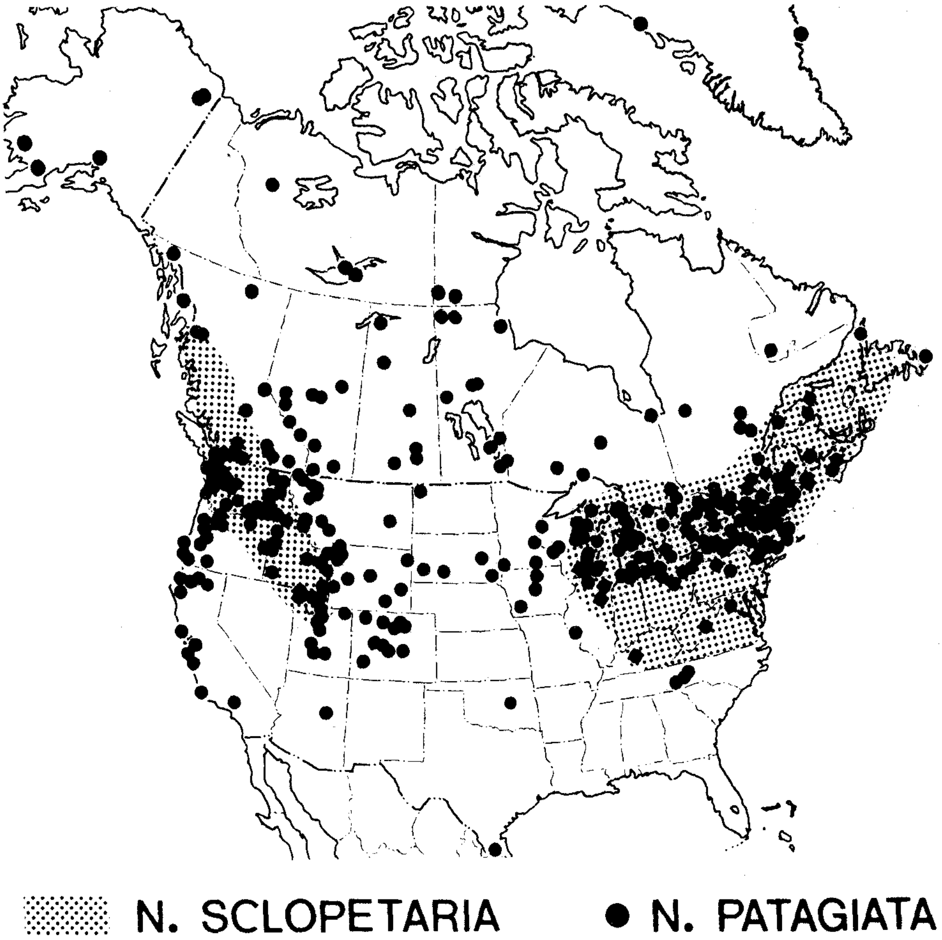


Figure 1.—Ranges of *Larinioides sclopetaria* and *Larinioides patagiata*, modified from Levi (1974).

found in Eurasia, this study focuses only on the specimens collected in North America. Here, the ranges of both species are completely encompassed by that of *Larinioides cornuta* (Clerck). *Larinioides sclopetaria* and *L. patagiata* have regions of sympatry on both the east and west coasts and an area of allopatry for *L. patagiata* between (Fig. 1).

Both the sizes and times of maturation of these two species overlap. *Larinioides sclopetaria* males have a carapace length of 3.7-4.2 mm and females a carapace length of 3.9-4.3 mm; *L. patagiata* has size ranges of 2.9-3.8 mm and 2.5-4.0 mm, respectively (Levi 1974). Size overlap can also be documented in specific sympatric regions (blocks A-D, Fig. 4) of each species range. Within each region, the smallest *L. patagiata* males had palpal femur and cymbium lengths that were 0.8-1.0 times those of the smallest *L. sclopetaria* males and the largest *L. patagiata* males had values that were 0.44-0.80 times those of the largest *L. patagiata* males.

These two species show no evidence of temporal isolation. In Connecticut, mature *L. sclopetaria* and *L. patagiata* can be found throughout the year (Kaston 1948). *Larinioides sclopetaria* is more commonly found around buildings than is *L. patagiata*, although Kaston (1948) notes that the former species is also found

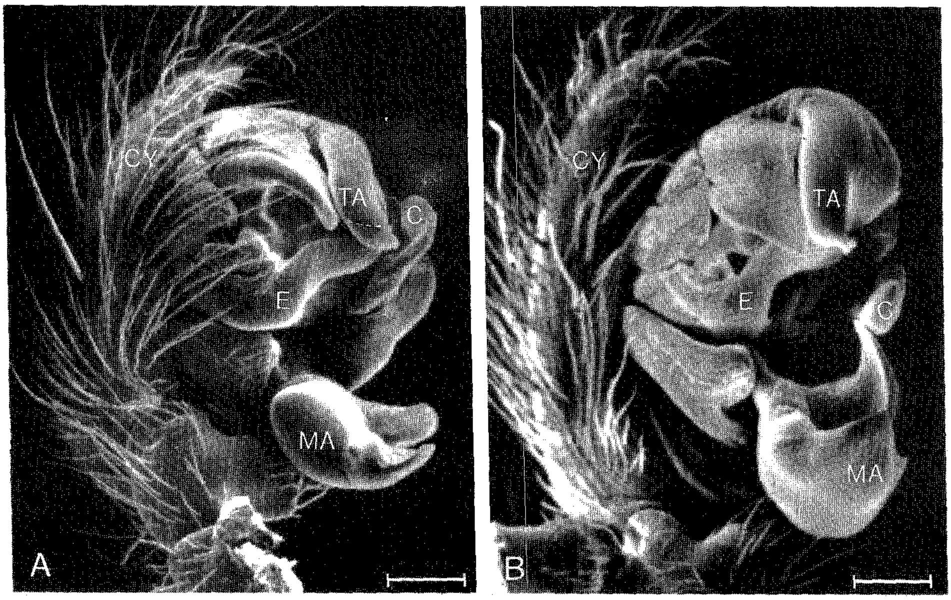


Figure 2.—Scanning electron micrographs of *Larinioides sclopetaria* (A) and *Larinioides patagiata* (B) left male pedipalps in ventral view. TA = terminal apophysis, C = conductor, CY = cymbium, E = embolus, MA = median apophysis. Scale bars each represent 200 μm.

on bushes and rocks near streams. This observation could suggest either that *L. sclopetaria* has a greater tolerance or is a more opportunistic species or that it prefers a different habitat than *L. patagiata*.

Distributional history.—Although both *L. sclopetaria* and *L. patagiata* are sympatric in Europe, Levi (1974) suggests that, due to its frequent association with man-made structures, *L. sclopetaria* may have been introduced into North America (*L. patagiata's* native status is unquestioned). However, he also notes that in Europe *L. sclopetaria* is also most commonly found on buildings. To evaluate this species' distributional history, we employed methods similar to those used by Nyffeler et al. (1985). This involved examining and plotting the collecting localities and dates of museum specimens of both species to detemine if there was convincing evidence for the range expansion of *L. sclopetaria*.

Measurements.—Sclerite measurements were taken at 50X using a dissecting stereo microscope fitted with an ocular micrometer scale. Values were recorded to the nearest half unit, providing a resolution of 10 μm. Only measurements of sclerites which could be positioned consistently and had well defined boundaries were used. In order to determine which palpal indexes could be accurately taken, three duplicate sets of preliminary measurements were taken on five males of the same species over a period of ten days.

Four types of measurements were used in this preliminary study: overall spider size (carapace length and first femur length), overall palp size (cymbium height and width), sclerite dimensions, and distances separating one sclerite from another. Coefficients of variation for all repeated measurements of the same species except the latter were less than 0.13, indicating that only sclerite separation could not be consistently measured.

In males of both species, one measurement (first femur length) of spider size and 16 palpal measurements were taken. First femur length (FFL) was measured

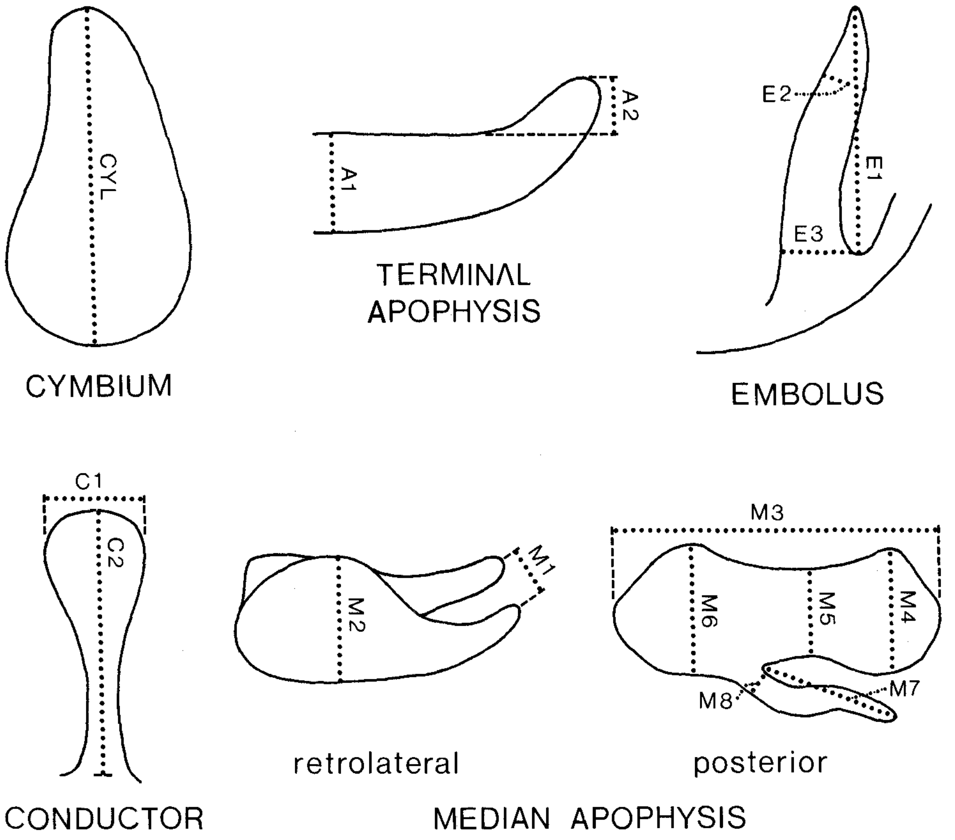
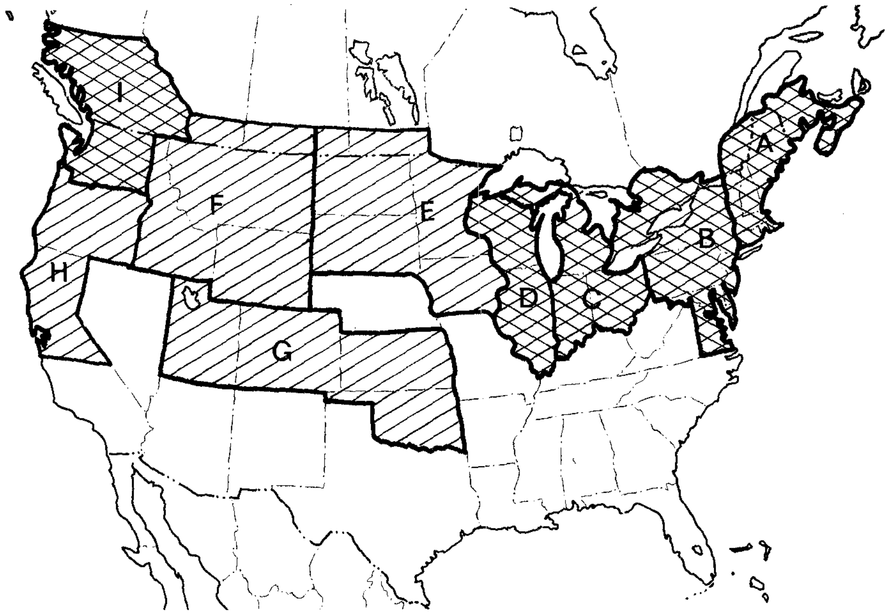


Figure 3.—Male palpal sclerites (not to scale), as oriented for measurement, and the features measured.

retrolaterally with the leg oriented perpendicular to the axis of measurement. Palpal characters (Figs. 2 and 3) were measured on the left pedipalp after it was removed from the body (unless the right pedipalp had been removed by a previous examiner). For the following palpal measurements, the dimensions being measured were oriented perpendicular to the axis of observation (directions refer to palpal orientation): cymbium length (CYL), prolateral view; palpal femur length (PFL), prolateral view; conductor width (C1) and length (C2), retrolateral view; terminal apophysis width (A1), apical (anterior) slightly retrolateral view (In *L. sclopetaria*, the greatest width measurement was taken; in *L. patagiata*, width was measured where the edges of the apophysis become parallel to one another.); curvature of the terminal apophysis (A2 - the distance from the main body beyond which the curved end projected), apical and slightly retrolateral view; embolus length (E1 - from the tip to the point where the base was curved on itself), anterior third width (E2), and basal width (E3), prolateral and ventral views, perpendicular to axis of measurement in an anterior-posterior direction and rotated laterally until the point where the base curved back on itself began to be eclipsed; median apophysis projection separation (M1) and depth (M2), prolateral and perpendicular to the axis of measurement in a lateral direction, rotated anterior-posteriorly until the main body just covered the upper edge of the bottom projection; median apophysis length (M3), upper ¼ width (M4),



BLOCK	A	B	C	D	E	F	G	H	I
L. SCLOPETARIA ▧	44	41	40	26	0	1	1	0	0
L. PATAGIATA ▨	30	28	25	8	14	60	22	16	14
TOTAL	74	69	65	34	14	61	23	16	14

Figure 4.—Range "blocks" and the number of specimens from each.

central width (M5), basal width (M6), and projection length (M7) and width (M8), posterior and slightly ventral, then rotated until the gap between the two projections and the main body was just barely eclipsed.

All statistical tests on these measurements were performed using SAS V (SAS Institute, Inc., SAS Circle, PO Box 8000, Cary, NC, 27511-8000).

Establishing range blocks.—Using state lines as boundaries, species ranges were divided into subunits (blocks) shown in Fig. 4. These blocks had similar areas and, except for the western blocks of *L. sclopetaria*, were represented by sufficient museum specimens to permit statistical tests. The scarcity of western *L. sclopetaria* males (Fig. 4) does not preclude comparisons of *L. patagiata* in areas of allopatry and sympatry with *L. sclopetaria*, as established by female specimens of the latter species.

Preliminary tests.—Before comparative statistical tests were performed, three preliminary steps were necessary. First, the normality of all measurements was tested with the Kolmogorov goodness-of-fit test to assure that assumptions of the statistical tests were met. Second, discriminate analysis was used to determine how well specimens of each species could be assigned to the correct geographical block. Only if most of the specimens from a block can be assigned to that block on the basis of palpal features alone, are the proposed blocks appropriate for this study. Third, *F*-tests comparing variability between the population and state levels, and the state and block levels were performed to determine if, as hypothesized, variability was greater in more inclusive units. If this is found to be correct, it means that blocks can be analyzed without being biased by overly large population or state variation.

Test of clinal variation.—We examined geographic variation because the patterns of variation within a species must be understood in order to avoid the problem of interpreting clinal variation as support for the mechanical isolation hypothesis. A large difference in mean measurement values between regions of sympatry and allopatry could actually be due to clinal variation, rather than the effect of range overlap.

To reveal less conspicuous, but congruent patterns in sclerite size differences indicative of clinal variation, we calculated sclerite measurement means for each geographic block (Fig. 4). For *L. patagiata*, this created a matrix of nine rows (blocks) and fifteen columns (one for each sclerite measurement except those measuring overall size - FFL, CYL, PFL) and for *L. sclopetaria*, a matrix of four blocks (the other two blocks included only one specimen each and were excluded from this analysis) and fifteen sclerites was constructed. From this matrix, we determined the number of sclerites for which each block had the highest value, the next highest value, etc., until the ninth lowest position was reached. These ranking variables were compiled into a summary table and from these, consensus rankings (Table 3) for each species were derived. These rankings were designed to detect any continuous trends in mean sclerite measurements across the ranges of the two species. If sclerite measurements show east-west or north-south clinal variation, consensus rankings will order the blocks of each species into a logical, east-west or north-south geographical sequence. However, this approach is not likely to detect other patterns, such as changes in sclerite values from the center of a species' range to its periphery or regional differences due to altitude or precipitation differences.

Test of character displacement.—We evaluated evidence for character displacement by comparing the means and variances of adjacent geographic blocks using *t*-tests and *F*-tests, respectively. When variances differed significantly ($P < 0.05$), *t*-tests for unequal variances were used.

RESULTS

Preliminary tests.—The Kolmogorov goodness-of-fit test shows the data to be normally distributed ($P \leq 0.05$). Discriminate analysis of block divisions (Table 1) shows a high percentage of specimens correctly identified, indicating that, although arbitrary, these block divisions are appropriate ones for pooling the data. Tests of variability between populations and states, and states and blocks showed that variability was significantly greater ($P < 0.05$) in more inclusive units.

Distributional history.—Museum specimens of both *L. sclopetaria* and *L. patagiata* date to the 1860's. Nothing about the pattern of acquisition of additional specimens chronicles an expanding range for either species. Specimens of both species collected in the eastern United States and Canada between 1864 and 1885 fully occupy the present-day eastern range of *L. sclopetaria* (Fig. 5). Those added during the next two decades simply increase the density of points within this range (Fig. 5), and do not, as Nyffeler, et al. (1986) found for the

Table 1.—Discriminant analysis of palpal features among geographic blocks. Letters in parentheses refer to those blocks into which the most frequently misidentified specimens were placed.

Species	Block	No.	% Identified correctly	Highest % identified incorrectly
L. sclopetaria	A	44	75	14 (B)
	B	41	90	5 (A & C)
	C	40	73	12 (A)
	D	26	92	4 (A & B)
L. patagiata	A	30	77	13 (E)
	B	28	86	11 (D)
	C	25	92	4 (D & E)
	D	8	100	0
	E	14	79	21 (D)
	F	60	75	7 (B & G)
	G	22	90	5 (E & F)
	H	16	88	12 (E)
	I	14	100	0

introduced species, *Steatoda bipunctata* (L.); depict a range expansion from the Northeastern coast of the United States inland and along the St. Lawrence River. Likewise, the number of new specimens of each species added to museum collections and the number of new state records resulting from this increase (Table 2) does not portray the expansion of *L. sclopetaria*. In fact, these data suggest that it is *L. patagiata* whose range expanded, a misconception explained by an increase in the number of specimens collected from the western states.

Test of clinal variation.—For each species, the consensus ranking (Table 3) lists in order the block that has the greatest number of sclerites placing it in the first, second, third, etc. position. The percentage of sclerites which contributed to the ranking of blocks was never much greater than 50% and averaged about 39%, indicating that no strong consensus appeared in the ranking. Additionally, the resulting consensus ranking shows no consistent trends of east-west or north-south clinal variation in either species.

Test of character displacement.—Significant intraspecific differences in the means of 0-8 palpal sclerite measurements were observed between adjacent blocks of both *L. sclopetaria* and *L. patagiata* (Tables 4 and 5). However, the pattern of sclerites that differed changed from block to block. In the eastern United States

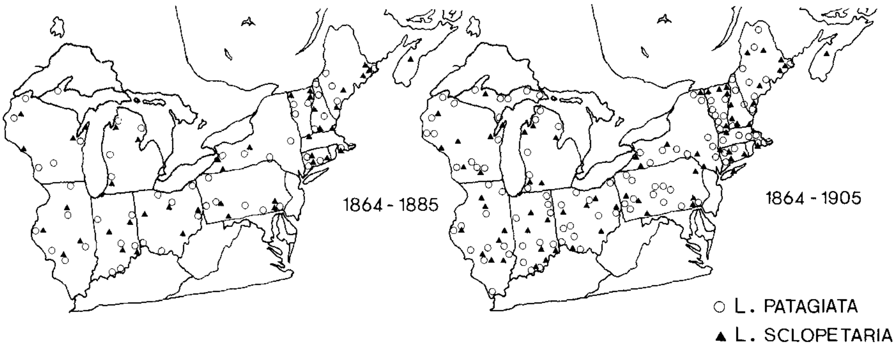


Figure 5.—Early distributional records for *Larinioides patagiata* and *Larinioides sclopetaria*.

Table 2.—A historical summary of the number of new specimens and new state records of *Larinioides patagiata* and *Larinioides sclopetaria*.

	Number of specimens		Number of new state records	
Time period	*L. patagiata*	*L. sclopetaria*	*L. patagiata*	*L. sclopetaria*
1864-1875	7	4 (36%)	(3)	(2) (40%)
1876-1885	12	9 (43%)	2	3 (60%)
1886-1895	0	5 (100%)	0	0 —
1896-1905	19	4 (17%)	4	3 (43%)
1906-1915	21	24 (53%)	4	2 (33%)
1916-1925	16	5 (23%)	5	1 (17%)
1926-1935	147	18 (11%)	9	2 (18%)
1936-1945	201	17 (8%)	6	0 (0%)
1946-1955	183	14 (7%)	3	0 (0%)
1956-1965	96	3 (3%)	3	1 (25%)
1966-1975	39	19 (33%)	0	3 (100%)

only M4, the median apophysis's upper width, differed significantly ($P < 0.05$) between blocks D and E of *L. patagiata* where the lock-and-key mechanism predicts the greatest difference. In contrast, in blocks A through D where *L. patagiata* and *L. sclopetaria* are sympatric, 1-6 *L. patagiata* sclerites differ significantly between adjacent blocks. A significant overall *L. patagiata* size (FFL) difference appears only between blocks A and B, indicating that the interpretation of these results is not confounded by interblock differences in spider size.

The lock-and-key mechanism predicts that in the West the means of more *L. patagiata* sclerites should differ between block I, which this species shares with *L. sclopetaria*, and adjacent allopatric blocks F and H than between blocks E-H where *L. patagiata* alone is found (Fig. 4). Table 5 shows that this is not the case. In *L. patagiata*, only A2, the terminal apophysis' distal curvature, differs significantly ($P < 0.05$) between blocks I and F and none of its sclerites differ

Table 3.—Consensus ranking of geographic blocks. This table lists in order the block that has the greatest number of sclerites placing it in the first, second, third, etc. position.

Species	Rank	Block	% of Sclerites contributing to block rank
L. patagiata	1	G	53
	2	F, I	20
	3	F, H	33
	4	A, F, H	27
	5	A	33
	6	C	47
	7	B	40
	8	B, D	27
	9	E	40
L. sclopetaria	1	A	53
	2	A, D	33
	3	B	47
	4	C	67

Table 4.—Male *Larinioides sclopetaria* first femur and palpal sclerite means (in μm) and (standard deviations) by geographical block. A "*" appears between blocks whose means differ significantly ($p \leq 0.05$) and a "+" between blocks whose variances differ significantly ($p \leq 0.05$). Blocks F and G were each represented by only a single specimen.

	BLOCK								
Feature	A		B		C		D	F	G
FFL	5852	+	5802	*	5418	*	6024	7470	7802
	(989)		(689)		(739)		(873)		
CYL	1413	+	1325	+	1300	+	1348	1640	1640
	(341)		(181)		(72)		(133)		
C1	253		248	+	238	*	252	300	280
	(19)		(16)		(26)		(23)		
C2	514	+	511	*	480		499	590	600
	(59)		(41)		(48)		(66)		
A1	185	+	180	+	186		187	240	200
	(24)		(17)		(23)		(20)		
A2	52	*	43		48		53	40	100
	(21)		(16)		(18)		(22)		
E1	328	+ *	314	+	305	*	321	380	400
	(35)		(17)		(31)		(27)		
E2	108	+ *	99	+	102	*	111	120	120
	(19)		(10)		(15)		(14)		
E3	135	*	121	+ *	143		145	120	140
	(15)		(14)		(25)		(21)		
M1	131	*	125		124		130	140	120
	(13)		(12)		(14)		(14)		
M2	242	*	232		230	*	243	260	260
	(19)		(19)		(24)		(18)		
M3	623	+	608		606		623	740	680
	(49)		(35)		(44)		(56)		
M4	241		236		229	*	243	240	240
	(26)		(24)		(26)		(26)		
M5	220		214		212		216	230	220
	(19)		(14)		(18)		(22)		
M6	306		313		306		305	320	300
	(31)		(24)		(24)		(28)		
M7	229		226	*	204	*	223	280	280
	(32)		(30)		(33)		(37)		
M8	73		71		66		70	80	100
	(12)		(13)		(15)		(16)		
PFL	804		782		766		816	1000	1000
	(86)		(73)		(92)		(113)		

significantly between blocks I and H. In contrast, 3-8 sclerites differ significantly between allopatric blocks E-H.

Likewise, intraspecific differences in variances do not support predictions of the lock-and-key mechanism that greater variance should be permitted in allopatric than sympatric regions. The variances of 1-4 (mean 2.3) *L. patagiata* sclerites differs significantly ($P < 0.05$) between adjacent allopatric blocks A-D, as compared with 1-4 (mean = 2.5) that differ between adjacent sympatric blocks E-H (Table 5). The variance of only two sclerites differs significantly between sympatric block D and block E, where *L. patagiata* alone is found. In the West, the variances of 0-2 *L. patagiata* sclerites differs between sympatric block I and adjacent allopatric blocks F and H. None of these observations lends support to the lock-and-key mechanism.

Table 5.—Male *Larinioides patagiata* first femur and palpal sclerite means (in μm) and (standard deviations) by geographical block. A "*" appears between the blocks whose means differ significantly ($p < 0.05$) and a "+" between the blocks whose variances differ significantly ($p < 0.05$).

	BLOCK										
Feature	A	B	C	D	E	F	G	H	I	F&H	F&I
FFL	4000	* 3666	3745	3920	3676	3689	3799	3577	3640	—	—
	(333)	(336)	(480)	(263)	(321)	(377)	(364)	(358)	(389)		
CYL	1335	* 1244	1251	1285	1214	+ * 1348	+ * 1422	* 1341	1341	—	+
	(85)	(76)	(89)	(99)	(128)	(86)	(130)	(104)	(127)		
C1	196	* 183	186	180	184	* 213	216	208	216	—	—
	(20)	(21)	(26)	(15)	(23)	(21)	(26)	(18)	(19)		
C2	535	* 507	511	505	495	* 548	566	+ * 525	542	+ *	—
	(42)	(41)	(42)	(54)	(52)	(55)	(59)	(35)	(40)		
A1	159	+ * 140	+ 146	139	149	145	147	146	135	—	—
	(31)	(19)	(31)	(24)	(19)	(26)	(33)	(21)	(18)		
A2	28	* 21	23	+ 50	+ 19	23	24	+ 21	19	+	+ *
	(13)	(9)	(11)	(62)	(10)	(12)	(14)	(7)	(5)		
E1	310	299	304	287	304	* 338	354	347	339	—	—
	(31)	(34)	(23)	(35)	(28)	(31)	(43)	(28)	(31)		
E2	86	82	83	76	78	* 92	93	89	94	—	—
	(12)	(12)	(11)	(7)	(12)	(12)	(16)	(13)	(14)		
E3	100	+ 93	* 105	97	87	* 102	+ 108	+ 106	101	—	—
	(20)	(14)	(12)	(17)	(12)	(14)	(24)	(14)	(12)		
M1	212	206	203	210	197	* 218	* 246	* 216	208	—	—
	(28)	(20)	(26)	(28)	(37)	(30)	(25)	(24)	(20)		
M2	236	226	213	* 242	219	229	234	* 209	213	*	—
	(34)	(37)	(32)	(25)	(30)	(29)	(28)	(29)	(31)		
M3	481	+ 469	480	492	447	475	+ * 506	485	504	—	—
	(69)	(30)	(36)	(32)	(60)	(70)	(40)	(53)	(52)		
M4	186	177	171	* 199	* 177	176	* 193	199	186	*	—
	(24)	(29)	(26)	(22)	(20)	(29)	(28)	(37)	(25)		
M5	335	* 306	318	+ 315	+ 321	334	348	+ 340	320	+	+
	(35)	(31)	(30)	(60)	(32)	(50)	(56)	(29)	(28)		
M6	272	265	278	275	253	* 283	287	290	287	—	—
	(29)	(38)	(39)	(45)	(34)	(38)	(39)	(24)	(32)		
M7	492	+ 479	466	471	461	* 513	527	524	529	—	—
	(90)	(49)	(57)	(70)	(53)	(51)	(51)	(59)	(38)		
M8	72	* 94	80	74	77	+ 97	93	97	101	—	—
	(21)	(22)	(28)	(19)	(39)	(26)	(30)	(19)	(19)		
PFL	697	* 626	653	676	638	673	+ 711	* 644	673	—	—
	(51)	(55)	(64)	(38)	(64)	(57)	(83)	(64)	(77)		

DISCUSSION

In the absence of a clearer understanding of palpal sclerite function, the mechanical isolating hypotheses predicts that, when adjacent blocks of a species range are compared, a greater number of sclerites should differ between a sympatric and an allopatric block than between two sympatric blocks. By failing to show this difference, this study fails to demonstrate the character displacement predicted by the lock-and-key mechanism. Even between sympatric blocks, there is a shift in the sclerites whose means differ significantly, making it difficult to argue that those *L. patagiata* sclerites that differ between regions of sympatry and allopatry (blocks D and E and blocks I and either F or H) are of greater importance in species isolation.

There are several levels of sclerite function: general orientation, alignment, and physical coupling. The last of these has the greatest potential to function in reproductive isolation. Since the exact function of several of the sclerites is unknown, we made an effort not to weight any sclerite beyond the limits of our ability to measure it consistently. Additionally, several measurements were taken on each sclerite to avoid neglecting any feature which might play a role in the actual coupling of the palp with the female's epigynum. Considering the large number of measurements taken from each specimen, a number of parameters involved in palp coupling were almost certainly analyzed. Two of the structures measured (the embolus and the conductor) are known to be directly involved in coupling (Grasshoff 1973a, b; Shear 1967). Therefore, the failure of this study to find evidence for character displacement cannot be dismissed on the grounds that it analyzes structures not involved in the coupling process.

This study emphasizes the danger inherent in establishing subspecies on the basis of differences in a few characters. Although blocks were established as arbitrary units for purposes of evaluating clinal variation, each meets the traditional definition of a subspecies as: "an aggregate of local populations of a species, inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species" (Mayr 1963). As spiders are not known to respect state boundaries, it is likely that these two species' ranges could be divided into other arbitrary units, each distinguished by significant differences in one or more sclerites.

The use of museum specimens resulted in data from different localities and dates being compiled into a single set. There are no assurances that specimens found sympatrically at the species level are actually sympatric at the more important population level. However, most of the collectors were not interested specifically in one or the other of these large, conspicuous species and both species probably had an equal chance of being collected if they occurred in the same area.

Several problems are encountered in studies of character displacement (Grant 1972), the first being clinal variation. Some studies that have claimed to show character displacement (e.g., Brown and Wilson 1956; Ficken et al. 1968) have later been refuted because the morphological changes attributed to character displacement could be explained by clinal variation (Grant 1972). Although this study detected no north-south or east-west clinal variation in either species, the block design it employs is not well suited to detect changes in sclerite values due to local differences in such factors as altitude or precipitation. However, because these local differences have a limited influence on a block's values, they are less likely to compromise the design of our study.

A second potential difficulty is that the historical events responsible for the present distribution of most species are not well documented. If two species evolved in isolation and then came into contact, there would be opportunity for character displacement to occur. If they first originated sympatrically and then one vacated part of the other's range, there would be opportunity for character release to occur in the vacated region. Two facts suggest that the present-day distributions of the species studied have existed long enough to permit their palpal sclerites to respond to such increased or relaxed selection pressure. First, as early as the 1860's both species' eastern ranges appear fully occupied (Fig. 5). Second, between adjacent blocks of each species (except F and G of *L.*

sclopetaria that were each represented by only a single specimen) there are significant differences between sclerite means and variances (Tables 4 and 5). However, there is the less likely possibility that these differences may result from a series of founder effects.

Three isolating mechanisms can function to increase the efficiency of mating where related species coexist: habitat isolation, ethological isolation, and mechanical isolation (Bush 1975). The third problem with studies of character displacement is that usually only one of these mechanisms is explored by the study. Grant (1972) found that in some cases what passed for displacement involving morphological characters was actually the result of habitat isolation (Ripley 1959; Ficken et al. 1968). No comparative ecological data were available for *L. patagiata* and *L. sclopetaria.* However, they may occupy slightly different habitats, even though collected from the same locality.

Ethological isolation can also explain the separation of species. However, Robinson and Robinson's (1980) comprehensive study of courtship shows that although most congeneric orb-weavers share a series of courtship behaviors, there is much intraspecific variation. In such cases, differences in the external genitalia of males and females would enhance reproductive isolation, even if they were not the sole or even principal mechanism.

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