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THE EFFECT OF ELEVATION ON THE DISTRIBUTION OF SIBLING SPECIES IN THE *SIMULIUM ARCTICUM* COMPLEX (DIPTERA: SIMULIIDAE)

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ABSTRACT.— At least 5 sibling species and an additional 11 cytotypes of the *Simulium arcticum* complex occur in Montana. Consequently, this speciose complex might allow study of environmental correlates with genetic differentiation. We used conventional methods of collection and cytogenetic analysis to study 1128 male larvae of the *Simulium arcticum* complex at 15 sites within 5 drainages in western Montana to test the hypothesis that distribution of siblings is associated with elevation. We sampled at the mouth, at the headwaters, and at an intermediate site to span the range of elevations within each drainage. We restricted our analyses to the most abundant taxa of the *S. arcticum* complex within our study area and observed a statistically significant presence of *S. apricarium* at low-elevation sites. *Simulium arcticum* IIL-18 appeared more frequently than expected at high elevation sites. *Simulium brevicercum* and *S. arcticum* sensu strictu appeared to be distributed randomly. We suggest potential causal reasons for these distributions including differential use of habitats along these elevational gradients.

Key words: black flies, chromosomal rearrangements, sibling species, elevation, sibling distributions.

Black flies (Diptera: Simuliidae) are often composed of cytologically differentiated and reproductively isolated sibling species (cytospecies), which can be observed when polytene chromosomes of larval salivary glands are analyzed (Rothfels 1979). The recognition of sibling species is based on sex-linked rearrangements, fixed chromosomal inversions, autosomal polymorphisms, the presence or absence of B chromosomes, or a combination of all these (Rothfels 1956, Rothfels et al. 1978, Shields and Procunier 1982, Newman 1983, Procunier and Shemanchuk 1983, Allison and Shields 1989). Often, sex-linked chromosomal rearrangements occur as paracentric inversions that are heterozygous in males and homozygous in females, with males of each sibling having a unique inversion. The numerous discoveries of reproductively isolated siblings of black flies among what were originally considered by conventional taxonomists as single morphospecies suggest that these chromosomal rearrangements promote or at least accompany the diversification process in these insects. The prevalence of sibling species among morphospecies of black flies provides an opportunity to investigate the causes of sibling distribution and to describe adaptation at the local level. Studies that test hypotheses about the causal determinants of distribution and attempt to explain reasons for the mechanisms of site selection by various black fly siblings are important and largely unexplored (Adler et al. 2004).

The *Simulium arcticum* complex of western North America is a case in point. We originally described 5 siblings (*S. arcticum*—standard, *arcticum* IIL-1, *arcticum* IIL-2, *arcticum* IIL-3, and *arcticum* IL-3.4) of this complex from Alaska and western Canada (Shields and Procunier 1982). These siblings have now been formally recognized as *S. brevicercum*, *S. arcticum* IIL-1, *S. saxosum*, *S. arcticum* sensu strictu (s.s.), and *S. negativum*, respectively (Adler et al. 2004). Two additional siblings, *S. arcticum* IIL-8.9/IIS-10.11 (*S. vampirum;* Adler et al. 2004) and *S. arcticum* IIS-4, were subsequently described from the Athabasca River drainage of Alberta, Canada, by Procunier and Shemanchuk (1983) and by Procunier (1984), respectively. Two more sibling species, *S. apricarium*, and *S. chromatinum*, have recently been recognized (Adler et al. 2004). An additional 16 taxa of the *S. arcticum* complex have been described as cytotypes (populations having unique sex-linked chromosomal rearrangements but for which there is yet no firm evidence of

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TABLE 1. Abundance (frequency of siblings/cytotypes) and distribution of the 4 major taxa of the *Simulium arcticum* complex at 15 sites (3 sites within each of 5 drainages). Elevations and ranges of water temperatures are given for each site.

Drainage and site	Elevation (m)	Temperature range (°C)	Frequency of siblings/cytotypes			
			S. *brevicercum*	*S. arcticum* s.s.	*S. apricarium*	*S. arcticum* IIL-18
BOULDER RIVER						
Bison Creek	1661	2–15	19	29	1	1
High Ore	1479	2–10	9	54	0	1
Cardwell	1260	7–15	0	0	54	0
LITTLE BLACKFOOT RIVER						
Kading C.	1720	8	7	2	0	1
Elliston	1478	7–12	111	61	0	40
Garrison	1263	9–18	4	3	56	0
TROUT CREEK						
Vigilante C.	1424	10–12	1	1	0	0
Mile 6.1	1349	10–12	25	8	0	0
Mouth	1193	8–13	32	8	85	0
FLINT CREEK						
Campground	1606	10–13	26	18	0	7
Philipsburg	1473	12–14	24	14	3	4
Hall	1275	5–16	20	8	9	2
CANYON CREEK/LITTLE PRICKLY PEAR						
Canyon Creek	1335	6–13	20	65	0	5
LPPC	1148	3–11	0	179	56	0
Mouth	1056	8–12	0	37	18	0

reproductive isolation; Adler et al. 2004, Shields unpublished data). With additional cytogenetic analyses, particularly where 2 or more cytotypes are found in sympatry, and additional morphological study, these types may prove to be good biological species as well.

The presence of 9 species and an additional 16 potential cytospecies within this complex suggests, not only extreme genetic diversity, but also the potential to study environmental correlates with the taxon-specific chromosomal variation. Given the diversity of types within our study area, we speculated that some might be adapted to higher mountainous regions and to colder temperatures in spring while others might be adapted to lowland/prairie locations where temperatures of streams are generally higher. We know that altitude affects species diversity in black flies given that 63 species have been found in the Sierra Nevada region of California, whereas only 28 species occur in the prairie region of North Dakota south to Oklahoma, an area 12 times larger than the Sierra Nevada region (Adler et al. 2004).

We studied the cytogenetics of the 4 most abundant taxa of the *S. arcticum* complex in west central Montana: *S. brevicercum, S. arcticum* s.s., *S. apricarium,* and *S. arcticum* IIL-18 (see Results and Discussion for a description of this taxon). Given the diversity within the *S. arcticum* complex here, we hypothesized that 1 or more types might be characterized by differential use of habitats along elevational gradients. Alternatively, if no ecological habitat selection were operative, the taxa would be randomly distributed throughout the 5 drainages.

METHODS

We sampled 15 collection sites in 5 different drainages (Table 1) at monthly intervals from April through August. We acknowledge that elevations for the 3 categories: high, intermediate, and low—are not consistent among drainages; rather, we emphasize elevational differences within drainages. All sites were sampled by a minimum of 3 researchers, who spent equal amounts of time (usually 45–60 minutes) at each site sampling all substrates including trailing vegetation, rocks, branches, and logs. All larvae of black flies, regardless of species, were removed with forceps and placed in a vial containing fresh, cold Carnoy's fixative (3 parts 100% ethanol: 1 part glacial acetic

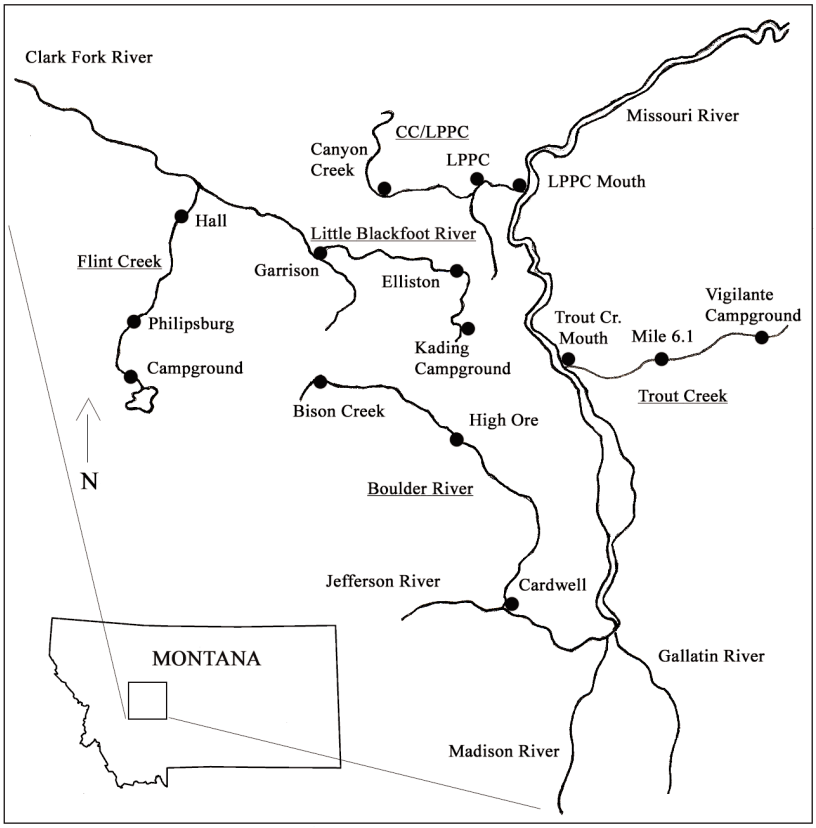

Fig. 1. Regional map of west central Montana indicating the 5 drainages and 15 collection sites at which abundance and presence-absence frequencies of *Simulium brevicercum*, *S. arcticum* s.s., *S. apricarium*, and *S. arcticum* IIL-18 were determined. The 5 drainages are underlined while enclosed circles designate the 3 sites (high, intermediate, and low) within each of these drainages at which larvae were collected. CC—Canyon Creek, LPPC—Little Prickly Pear Creek.

acid). Collection vials were placed on wet ice until we returned to the laboratory where the Carnoy's fixative was changed repeatedly until it appeared colorless (usually 4 changes). At each site we recorded temperature of stream water to the nearest degree centigrade and elevation to the nearest meter with a Suunto portable altimeter. The altimeter was calibrated to 1181 m above sea level (ASL) at the Helena International Airport before each collection run. Larvae were sorted to morphospecies in the laboratory, using the criteria of Currie (1986). Subsets of all sample collections in Carnoy's fixative are maintained in the G.F. Shields Simuliid Collection in the Department of Natural Sciences at Carroll College, Helena, Montana. Penultimate and ultimate instar larvae of the *S. arcticum* complex from each site were sorted and placed in a fresh vial of Carnoy's fixative. Polytene chromosomes of the salivary glands and gonads of each larva were stained in Feulgen (Rothfels and Dunbar 1953), and chromosome complements were scored for variation by comparing them to the standard chromosome maps for the *S. arcticum* complex (Shields and Procunier 1982, Adler et al. 2004). The latest progression of meiosis and the presence or absence of supernumerary or B chromosomes were determined from the same slides as those having polytene chromosomes. Since female larvae within the *S. arcticum* complex, excepting larvae of *S. saxosum* and *S. apricarium*, usually possess the standard chromosome sequence in each homologue, we were unable to assign females to specific siblings or cytotypes when they occurred sympatrically with other taxa of the *S. arcticum* complex. Consequently, we restricted our analyses to males.

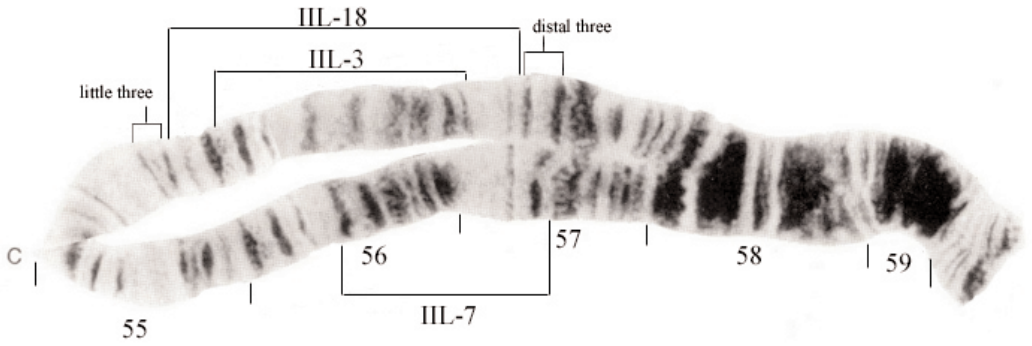

Fig. 2. The basal portion of the long arm of chromosome II of the *Simulium arcticum* complex showing proximal (left) and distal (right) breakpoints in brackets of the sibling-specific, sex-linked chromosomal rearrangements for taxa of this study. *Simulium brevicercum* possesses the standard chromosome sequence. *Simulium arcticum* s.s., *S. apricarium*, and *S. arcticum* IIL-18 are characterized by the IIL-3, IIL-7, and IIL-18 inversions, respectively. Numbers below the chromosome indicate sections (55–59) of this portion of the IIL chromosome. C—centromere.

TABLE 2. Mean abundances and standard errors (in parentheses) of each sibling/cytotype at each elevation.

Elevation	*S. brevicercum*	*S. arcticum* s.s.	*S. apricarium*	*S. arcticum* IIL-18
High	14.6 (4.6)	23.0 (11.7)	0.2 (0.2)	2.8 (1.4)
Medium	33.8 (19.9)	63.2 (30.8)	11.8 (11.1)	9.0 (7.8)
Low	11.2 (6.4)	11.2 (6.6)	44.4 (13.8)	0.4 (0.4)

For each of the 3 elevational categories, we summarized presence-absence statistics for each of the taxa and mean abundance for each of the siblings and cytotypes. Then we used analysis of variance (ANOVA) to test whether mean abundance of each sibling differed across the 3 elevations. We also used contingency analysis to test whether presence/absence for each taxon was associated with elevation (Zar 1984).

RESULTS AND DISCUSSION

Cytological Description of *S. arcticum* IIL-18

Simulium arcticum IIL-18 was originally described by Santoro (2004) from the Canyon Creek site (Fig. 1). The proximal breakpoint of this inversion in the long arm of chromosome II is just after the 3rd band of the "little three" in section 55, and its distal breakpoint is before the "distal three" in section 57 (Fig. 2). Meiosis in IIL-18 advances to anaphase of 1st division and this cytotype has no B chromosomes. Linkage to the Y chromosome is apparently complete because all 63 larvae having this specific inversion were males. *Simulium arcticum* IIL-18 has been found only in Granite, Jefferson, Lewis and Clark, and Powell Counties of west central Montana. It is the 3rd most abundant taxon (17.1% of males) at the Elliston site of the Little Blackfoot River.

Distributional Analysis of Major Taxa

A total of 2839 larvae of the *S. arcticum* complex were analyzed. Of these, 720 were of siblings and cytotypes that were either absent or too infrequent at 1 or more sites to analyze here. Of the 2119 remaining larvae, 991 were female; thus, our analysis was based on 1128 male larvae of *S. brevicercum*, *S. arcticum* s.s., *S. apricarium*, and *S. arcticum* IIL-18. Frequency distributions of these taxa at each of the 15 collection sites are shown in Table 1. Mean abundances for these taxa at each of the 3 elevational categories indicated that most larvae of *S. apricarium* (44.4) occurred at low elevations while most larvae of *S. arcticum* s.s. (63.2), *S. brevicercum* (33.8), and *S. arcticum* IIL-18 (9.0) occurred at intermediate elevations (Table 2). Analysis of variance testing for

TABLE 3. Analysis of variance testing for the effects of elevation on abundance. The degrees of freedom for the effect and mean square error equal 2 and 12, respectively, for all dependent variables.

	F[a]	P
S. brevicercum	0.98	0.405
S. arcticum s.s.	1.97	0.182
S. apricarium	5.02	0.026[b]
S. arcticum IIL-18	0.94	0.416

[a]Dependent variable = abundance.
[b]Significant.

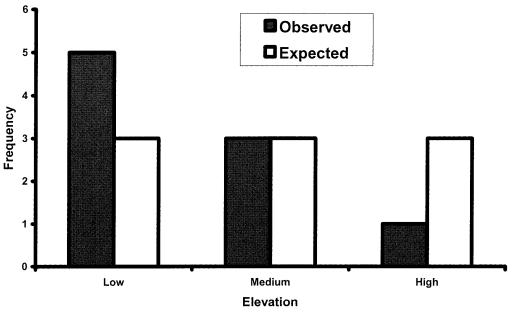

Fig. 3. Observed and expected frequencies of *Simulium apricarium* at low-, medium-, and high-elevation sites among the 15 sites within 5 drainages studied.

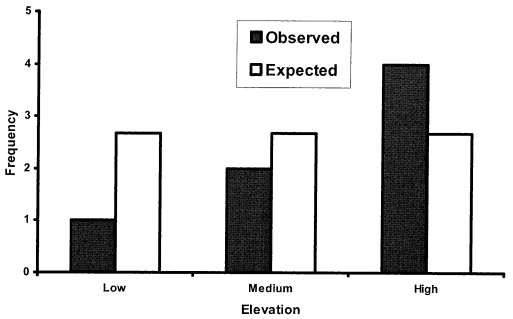

Fig. 4. Observed and expected frequencies of *S. arcticum* IIL-18 at low-, medium-, and high-elevation sites among the 15 sites within the 5 drainages studied.

the effects of elevation on abundance indicated that *S. apricarium* abundance was significantly influenced by elevation (Table 3). Furthermore, *S. apricarium* abundance was significantly higher (post hoc Tukey HSD test: $P = 0.025$) at low-elevation sites than at high-elevation sites.

Contingency analyses testing for differences in presence/absence of types across the 3 elevations indicated a significantly greater proportion of *S. apricarium* at low elevations (Fig. 3) and a significantly greater proportion of *S. arcticum* IIL-18 at high elevations (Fig. 4). The results support our original hypothesis that there may be elevational separation in larval habitats among the taxa. Among the 1128 larvae analyzed, only 1 individual of *S. apricarium* was found at any of the 5 high-elevation sites. Alternatively, *S. apricarium* was present at all low-elevation sites and abundant at 3 of these. The single *S. apricarium* male at Bison Creek (1661 m ASL) was found late in the season (15 August) and in 15°C water. Thus, an interplay between elevation and water temperature may influence distribution of this sibling. The formal epithet *S. apricarium* used by Adler et al. (2004) literally means "of the open" and refers to its presence in low-elevation prairie habitat. Among the 1128 male larvae identified chromosomally to sibling, only 4 *S. apricarium* were observed above 1475 m, a result indicating a strong presence at low-elevation sites for this sibling.

Simulium arcticum IIL-18, though abundant only at the Elliston site and apparently localized in its distribution, was present at high elevations in greater-than-expected frequencies. Based on this finding, we suggest that S. arcticum IIL-18 is adaptated for high elevations and the consequent colder temperatures (5°C; Shields unpublished data). *Simulium arcticum* IIL-18 did not occur among the larvae analyzed from the 3 sites of the Trout Creek drainage, and only 2 individuals of this cytotype occurred in the entire Boulder River drainage, albeit at intermediate and high elevations. Thus, we are reluctant to speculate on the ecological distribution of this rare cytotype. With the exceptions of Cardwell (low elevation) and Little Prickly Pear Creek and its mouth (intermediate and low elevations, respectively), *S. brevicercum* was found at 12 of the 15 sampled sites and *S. arcticum s.s.* was absent only at the Cardwell site. Thus, we suggest that these 2 siblings may not be limited by elevation or temperature.

Distributions of the 4 taxa of the *S. arcticum* complex in this study could also be influenced by their histories. Present distributions of the 4 taxa differ greatly. *Simulium brevicercum* has a large distribution from Alaska south

to Alberta, Montana, Utah, and California. *Simulium apricarium* occurs in 9 states of the Rocky Mountain region primarily to the south and west of Montana, while *S. arcticum s.s.* is distributed in an area about one-third that size in Idaho, Montana, Alberta, and British Columbia primarily northwest of Montana (see Adler et al. 2004:814–822 for information on present distributions). Possibly, these siblings have come into relatively recent contact after prolonged adaptation to different environmental conditions. Alternatively, *S. arcticum* IIL-18 has been found at only 4 sites in a 4-county region in west central Montana. *Simulium arcticum* IIL-18 and 10 other cytotypes have very limited distributions in Montana (Shields unpublished data). Unlike the broadly distributed siblings mentioned above, the distributions of cytotypes and their presence with other siblings and cytotypes suggest the possibility of an in situ origin. Although allopatric speciation cannot be ruled out, the distributions of all these cytotypes could also be explained by a sympatric speciation model similar to one suggested by Rothfels (1989).

Stream velocity and depth can be predictors of the distributions of black fly species (Adler and McCreadie 1997). Thus, *S. apricarium* may simply be adapted to slower moving streams while *S. arcticum* IIL-18 may be adapted to more rapidly moving waters descending from higher elevations. We cannot address this issue here because each of the 5 drainages studied was chosen only for its elevational relief, and velocity and depth were not measured.

It is more likely that eggs and larvae of *S. brevicercum, S. arcticum* s.s., and *S. arcticum* IIL-18 "drift" downstream during and after egg deposition rather than eggs or larvae of *S. apricarium* somehow moving long distances upstream. We know little about movement of eggs after they have been laid (Adler et al. 2004), but it has been estimated that larvae of some species may drift hundreds of kilometers downstream (Rubtsov 1964).

Santoro (2004) suggested that sibling group composition within the *S. arcticum* complex was similar at 2 sites along the Little Blackfoot River. While this may be true for limited study of 2 sites at similar elevations, it is not the case when multiple drainages including sites at various elevations are studied throughout the summer as shown here.

Distribution of siblings may also be influenced by availability of nutrients concentrated at outflows (Adler and Kim 1984, Wotton 1988, McCreadie and Colbo 1992). We collected samples in the Flint Creek drainage as it flows from Georgetown Lake, Granite County (1944 m ASL), throughout the summer of 2004 and found only *S. vittatum*, which inhabits lake outflows in western North America (Adler 1986, Ciborowski and Adler 1990). The Flint Creek Campground site, at which we found only *S. arcticum* types, is only 1.6 km downstream from the outlet of Georgetown Lake but 335 m lower in elevation. Possibly, a combination of the effects of accumulated nutrients at this lake outflow and elevation influence the distributions of both *S. vittatum* and members of the *S. arcticum* complex there.

Studies such as this may lead to the elucidation and importance of environmental cues that influence the location of egg-laying by female black flies. Based on our observations elevation may influence the distribution of *S. apricarium* and *S. arcticum* IIL-18 in west central Montana. Whether this distribution is determined by history, elevation, temperature, adaptation to local environments, or a combination of all of these factors must await additional study.

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