Western North American Naturalist 79(2), © 2019, pp. 148–158

Do cytotypes of black flies of the *Simulium arcticum* complex (Diptera: Simuliidae) arise from sibling species?

Gerald F. Shields[1,*] and D. Grant Hokit[1]

[1]*Department of Life and Environmental Sciences, Carroll College, Helena, MT 59625*

Abstract.—To better understand the evolutionary radiation of the *Simulium arcticum* complex of black flies (Diptera: Simuliidae), we compared the geographic distributions of present-day larvae to their sex-chromosome diversity. We used the 5 known data sets including collections and sex-chromosome analysis from 307 geographic locations of 31 taxa of approximately 20,000 larvae from throughout the geographic range of distribution of the complex, from Alaska, western Canada, and the western United States to southern California, Arizona, and New Mexico. Siblings (reproductively isolated in sympatry) have considerably larger geographic distributions than do cytotypes (not reproductively isolated in sympatry), suggesting that the former may have been in existence longer than the latter. *Simulium negativum* (the oldest member of the complex), *S. brevicercum* (standard noninverted sex chromosomes), *S. saxosum* (sex determination on the X chromosome), and *S. arcticum* s. s. (IIL-3) share geographic distributions with all other siblings. Notably, 21 of 22 cytotypes share geographic distributions within those of siblings. Cytotypes are almost always discovered within the geographic distributions of siblings, suggesting that the former might be arising sympatrically.

Resumen.—Para comprender mejor la radiación evolutiva del complejo *Simulium arcticum* de moscas negras (Díptera: Simuliidae), comparamos las distribuciones geográficas de las larvas actuales con la diversidad de sus cromosomas sexuales. Utilizamos cinco bases de datos conocidas, incluyendo colecciones y análisis de cromosomas sexuales de 307 zonas geográficas de 31 taxones, de aproximadamente 20,000 larvas a través del rango geográfico de distribución del complejo. Desde Alaska, el oeste de Canadá y el oeste de los Estados Unidos hasta el sur de California, Arizona y Nuevo México. Las especies hermanas (reproductivamente aisladas en simpatría) poseen distribuciones geográficas considerablemente más grandes que los citotipos (no reproductivamente aislados en simpatría), sugiriendo que los primeros pueden haber precedido a estos últimos. *Simulium negativum,* el miembro más antiguo del complejo, *S. brevicercum* (cromosomas sexuales estándares no invertidos), *S. saxosum* (determinación sexual en el cromosoma X) y *S. arcticum* s. s. (IIL-3) comparten sus distribuciones geográficas con todas las demás especies hermanas. Cabe destacar que 21 de los 22 citotipos comparten distribuciones geográficas con las de las especies hermanas. Los citotipos casi siempre se descubren dentro de las distribuciones geográficas de especies hermanas, indicando que los primeros podrían surgir en simpatría.

Black fly larvae (Simuliidae) possess informative polytene chromosomes in their salivary gland nuclei, and chromosomal rearrangements in sex chromosomes define taxa (Rothfels 1979a, Shields and Procunier 1982). Black flies are also of interest because initially described single morphospecies almost always result in descriptions of complexes of numerous cytospecies when their larval polytene chromosomes are studied (Rothfels 1956, 1979a, 1979b, 1981a, 1981b, Rothfels et al. 1978, Shields and Procunier 1982). Cytogenetic studies indicate that these complexes are composed of types whose sex chromosomes differ by unique paracentric chromosomal inversions, usually in males (Rothfels 1956, 1979a, 1989, Shields and Procunier 1982, Adler et al. 2004). Once complexes are identified through chromosome analysis, additional features of the biology of the taxa are described with the result that the complexes are resolved into good biological species (Adler et al. 2004). Current complexes that deserve additional study include the following: *Prosimulium ursinum, Simulium luggeri, S. noelleri, S. arcticum, S. paynei, S. virgatum,* and *S. trigonium* (Adler and Crosskey 2018). The total rises to more than 45 complexes worldwide (Adler personal communication).

Studies that report genetic types and their geographic locations are rare (Coyne and Orr 2004). Yet, such studies, though based on

*Corresponding author: gshields@carroll.edu

present-day distributions, might suggest origins of taxa, define the geographic limits of distributions, reveal relative ages of types (with the working assumption that taxa with large distributions might be old), suggest closest relatives, and indicate clusters of taxa adapted to specific locations.

Rothfels (1989) proposed a model for a sympatric mode of speciation for black flies and suggested that it involved changes in sex-chromosome polymorphisms that displayed linkage disequilibrium and that cytotypes shared ancestral polymorphisms and widely overlapping geographic distributions of related taxa. The Rothfels model was based, in part, on the then-available data for *S. arcticum*, so we decided to test the model with a much larger data set. Justification for this approach also relates to speciation models that suggest that chromososomal inversions might lead to speciation by reducing recombination between sex-determining alleles (Hoffman and Rieseberg 2008, Feder and Nosil 2009, Ayala et al. 2011, Guerrero et al. 2012). This may also be true for sympatric populations of mosquitoes whose DNA has been compared at the level of the genome (Love et al. 2016). Gavrilets (2014) has summarized our current understanding of speciation and has suggested that sympatric speciation is theoretically possible.

Twenty-two years of our own cytogenetic studies indicate that cytotypes (taxa within the *Simulium arcticum* Malloch complex that have unique sex chromosomes, but whose reproductive status with sympatric taxa has not yet been determined) are always discovered within the geographic distributions of sibling species (taxa reproductively isolated in sympatry). Indeed, all of the 16 cytotypes we have described within the *S. arcticum* complex occur within the geographic distributions of siblings (Shields 2013, Shields and Shields 2018). These observations were made sequentially as new taxa were discovered over time (Shields 2013), and it is the sum of these isolated observations that stimulated us to analyze all available information on *S. arcticum* to gain a better understanding of how this diversity occurred.

The *S. arcticum* complex consists of 9 described sibling species and at least 22 cytotypes (Shields and Procunier 1982, Adler et al. 2004, Shields 2013, Shields and Shields 2018; Shields unpublished data). This massive number of taxa within what was initially described as a single morphospecies (Malloch 1914) suggested that analysis of all available data may be informative.

Our extensive cytogenetic research on the *Simulium arcticum* complex in Alaska, western Canada, Montana, Idaho, and eastern Washington indicates that sex-linked chromosomal inversions appear early in the differentiation process before morphological change occurs (Shields and Procunier 1982, Adler et al. 2004, Shields 2013). Moreover, comparisons of mitochondrial and nuclear DNAs of all taxa of the complex suggest (1) that the group may be about 500,000 years old and (2) that *S. negativum* is the only member of the complex that is monophyletic in phylogenies and is morphologically unique (Adler et al. 2004, Conflitti et al. 2017). The radiation of the remainder of the complex is estimated at about 250,000 years old, yet these taxa are not monophyletic—that is, they are intermingled with one another in our phylogenies (Conflitti et al. 2017).

We combined 5 separate data sets and used modern methods of geographic analysis to describe distributions of the 31 taxa within the *S. arcticum* complex. We hoped to shed light on not only the origins and adaptations of all taxa within the complex but also the current distributions of taxa. If present-day distributions are suggestive of origins, several predictions might accompany this analysis: (1) if an allopatric mode of speciation is suggested, all or a majority of the geographic distributions should show no overlap with other siblings and cytotypes; (2) extensive overlap of distributions or distributions entirely within other distributions might suggest closest relatives and sympatric speciation; (3) older taxa should share distributions with many, if not all, siblings and cytotypes; and (4) sharing of distributions could indicate similar habitat preferences for chromosome taxa. Given our previous observations, we hypothesized that new types would be discovered within the geographic distributions of other previously described types within the complex. If this observation held true for the entire complex, it might argue for a sympatric mode of speciation.

Methods

Five separate data sets were included in our analyses: Alaska (Shields and Procunier

1982); Montana, Idaho, and eastern Washington (Shields 2013, 2014, Shields and Shields 2018; Shields unpublished data); throughout the range of *S. arcticum* (Conflitti et al. 2017); western Canada (Procunier unpublished data); and throughout the range of *S. arcticum* (Adler et al. 2004). The first 3 data sets were accompanied by GPS coordinates of collection sites and actual numbers of larvae for all taxonomic categories, whereas the last 2 data sets were located only to county of origin.

Locations for 307 sites were determined with varying accuracy. There were 96 sites associated with recent collections using a GPS that had an accuracy of ±5 m. There were 119 legacy sites with good location descriptions that we were confident were within 1 km of the actual collection location. Finally, 92 sites had poor location descriptions, often listing only a county. We used the centroid of the county location for these sites, resulting in a spatial accuracy of within approximately 100 km. Locations for each type, along with associated sibling species and cytotypes, were used to create an attribute table.

Attribute data were mapped and analyzed using ArcGIS Pro 2.2, similar to Swenson and Howard (2005). First, point features were created for each sibling species and cytotype. The geographic extent of each species and cytotype was defined using the minimum bounding geometry tool with the convex hull option to minimize the assumed geographic extent of each species/cytotype. It was not possible to use the minimum bounding geometry tool for cytotypes known from fewer than 3 locations. For these cytotypes, point and line features were created using the buffer tool and a buffer distance of 5 km. This is well within the known dispersal distance of individuals of this species complex (Adler et al. 2004). For all species and cytotypes, the resulting polygons were clipped using a mask of North America. Following this, the union tool was used to measure the contact between range extents of each pairwise combination of species and cytotypes. The number of contacts was quantified for each and categorized as a contact with a sibling species or with a cytotype. To create a more continuous surface feature, each contact polygon was converted to a raster surface using 1 km as the surface resolution. Finally, we listed the collection locations, number of collections, proportion of cytotypic area of distribution, and proportion of each cytotype in relation to other male chromosome taxa collected at the same site.

RESULTS

From 307 collection locations, we estimated the areas of the geographic distributions of nearly 20,000 larvae of 31 taxa (9 siblings and 22 cytotypes) from throughout the range of distribution of the *S. arcticum* complex (Fig. 1). The average area of distribution of siblings (1,755,467.7 km^2) is considerably larger than the average area of distribution of cytotypes (10,287.3 km^2; Tables 1, 2). *Simulium saxosum* and *S. negativum* have the largest areas of distribution (2,829,500 and 2,819,090 km^2, respectively; Table 2.). Though most geographic ranges of all siblings overlap with one another, they differ with respect to overlap (Table 2, Fig. 2). Four siblings, *S. negativum*, *S. saxosum*, *S. brevicercum*, and *S. arcticum* s. s., overlap with 8 other siblings (Table 2). Notably, *S. negativum* and *S. brevicercum* overlap with most cytotypes (n = 18; Table 2, Fig. 3). It is also notable that all cytotypes, except IIL-14, overlap with all other siblings (Fig. 3). Thus, the geographic distributions of 21 of 22 cytotypes are within the distributions of siblings. Characteristics of all cytotypes are enumerated in Table 3.

DISCUSSION

Areas of distribution of siblings (average 1,755,468 km^2) are considerably larger than areas of distribution of cytotypes (average 10,287 km^2), suggesting that the former may have been in existence longer than the latter. *Simulium saxosum, S. negativum, S. arcticum* IIL-1, and *S. brevicercum* have the largest areas of distribution, suggesting that they may be the oldest siblings. Notably, *S. negativum* is the basal lineage of the complex, having diverged from all other siblings an estimated 467,000 years ago based on our mitochondrial (COI, COII, cytb, and ND4) phylogenetic trees (pages 13 and 14 *in* Conflitti et al. 2017). Moreover, *S. brevicercum* has a large geographic distribution and occurs in all of the remaining clades of the same mitochondrial phylogenies, suggesting that it too may be ancient. That *S. negativum* is the only member of the complex whose sex is determined by a gene or genes in the long arm of chromosome I,

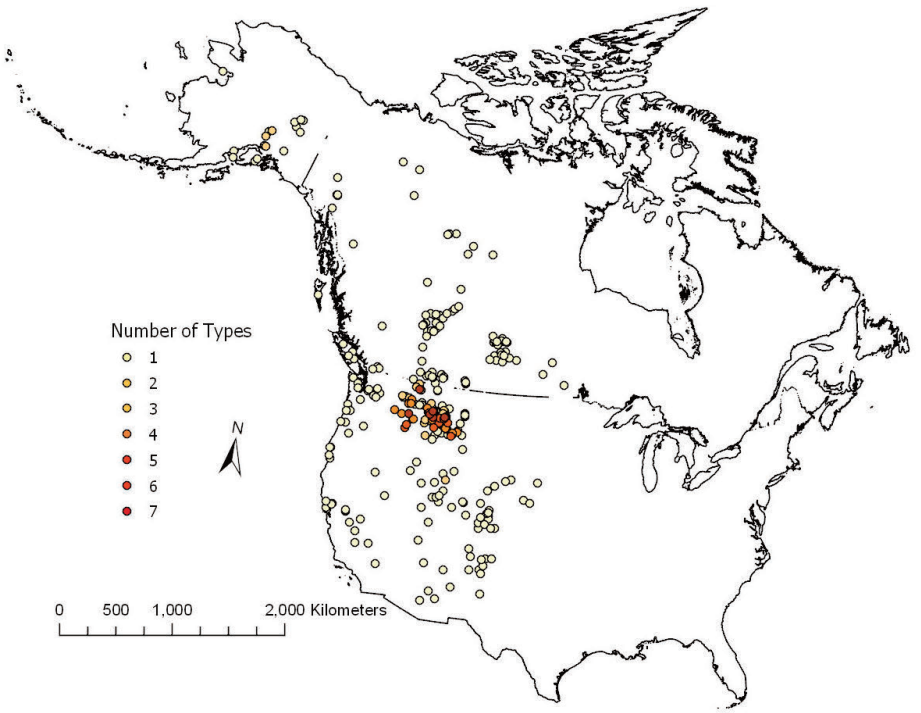

Fig. 1. Locations of collections and analysis of taxa of the *Simulium arcticum* complex.

while *S. brevicercum* has the standard (non-inverted) chromosome sequence in both sexes (Shields and Procunier 1982), additionally suggests the possibility that these taxa are basal to the group. It is possible that a *S. brevicercum*–like ancestor gave rise to all other members of the complex whose sex is determined in the long arm of chromosome II (page 12 *in* Conflitti et al. 2017). The geographic distributions of *S. negativum*, *S. saxosum*, *Simulium brevicercum*, and *S. arcticum* s. s. overlap with all sibling species of the complex. This might suggest that these taxa have a common origin and share preferences for similar habitats. *S. negativum* and *S. brevicercum* share distributional overlaps with the largest number of cytotypes (n = 18). Again, this may suggest an ancestor-derived relationship.

Conversely, 21 of the 22 cytotypes have very limited geographic distributions (39,902 to 8 km^2). This may suggest that they are relatively young, evolutionarily. This, and the facts that all cytotypes except IIL-14 share distributional overlaps with siblings and that half of them share geographic overlaps with a majority of siblings, may suggest that cytotypes evolve unique sex chromosomes based on paracentric inversions and are derived from siblings, possibly in a sympatric fashion. This pattern of cytotypes being discovered within the geographic distributions of siblings is a recurring theme of our cytogenetic research (Shields 2016) and a justification for the present research topic. Cytotypes share distributions with siblings almost twice as often as they do with other cytotypes, and only one (IIL-14) appears to be geographically isolated. Geographic isolation of a larger number of cytotypes would argue for allopatric speciation.

The IIL-14 cytotype is somewhat tentative, having been described only from a single collection (1 July 2001) at the Great Bear River as it exits Great Bear Lake in the Northwest Territories of Canada (Adler et al. 2004). This taxon and the area around it deserve more research.

The *S. arcticum* IIL-9 cytotype has an extensive geographic distribution (80,355 km^2) that is larger than that of the IIS-4 sibling. IIL-9 overlaps in distribution with 7 other siblings, 5 of which are shared by more than 71.4%. *Simulium arcticum* IIL-9 is very

Table 1. Statistics of geographic overlap for all taxa of the *Simulium arcticum* complex. One hundred indicates complete overlap; 0 indicates no overlap.

	IIL-2 Sax	IL-3.4 Neg	IIL-1	Y0 (IIL) Bre	IIL-7 Apr	IIL-3 ArcSS	IIL-11 Chr	LLS-10.11 Vam	IIS-4	IIL-9	IIL-13	IIL-15	IIL-10	IIL-79	IIL-19	IIL-18
IIL-2 Sax		69.4	55.1	79.7	8.8	71.7	9.5	26.2	100	94.5	100	34.6	1.4	100	100	24
IL-3.4 Neg			47.6	78.6	45.3	68.6	66.3	17.1	100	100	100	100	100	100	100	100
IIL-1				38.9	0.1	84.1	0	98.2	100	10.5	90.6	0	0	0	23.3	0
Y0 (IIL) Bre					31.4	54.2	31.5	5.8	93.4	100	99.4	100	78.6	100	100	100
IIL-7 Apr						7	96.7	0	0	71.4	10	100	100	67.2	76.2	100
IIL-3 ArcSS							1	71.9	100	100	99.5	100	75.9	83.1	100	100
IIL-11 Chr								0	0	2.8	0	0	0	25.1	0	0
LLS-10.11 Vam									64.4	0	0	0	0	0	0	0
IIS-4										0	0	0	0	0	0	0
IIL-9											24.9	31.4	3.3	62.1	100	35.3
IIL-13												3.8	0	0	49	0
IIL-15													41.4	0	74.3	87.8
IIL-10														0	0	41.9
IIL-79															0	0
IIL-19																0
IIL-18																
IIL-22																
IIL-6																
IIL-80																
IIL12																
IIS-15																
IIL-73.74																
IIL-17																
IIL-21																
IIS-12																
IIL-dup.																
IIS49_52																
IIL57_58																
IIL14																
IIL16																
IIL-68																

Table 1. Continued.

	IIL-22	IIL-6	IIL-80	IIL12	IIS-15	IIL-73.74	IIL-17	IIL-21	IIS-12	IIL-dup.	IIS49_52	IIL57_58	IIL14	IIL16	IIL-68
IIL-2 Sax	100	79.8	100	100	100	0	100	100	0	0	100	100	0	0	0
IL-3.4 Neg	100	76.3	100	0	100	100	100	100	100	100	0	100	0	0	100
IIL-1	62.6	3	0	0	100	0	0	0	0	0	0	0	0	100	0
Y0 (IIL) Bre	100	0	100	0	100	100	100	100	40.1	40.1	100	100	0	0	100
IIL-7 Apr	23	0	27.4	0	0	100	100	0	100	100	100	0	0	0	100
IIL-3 Arc SS	100	0	75.2	0	100	100	100	100	0	0	0	0	0	0	100
IIL-11 Chr	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
LLS-10.11 Vam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIS-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIL-9	77.6	0	25.3	0	6.3	0	100	73.4	0	0	0	0	0	0	0
IIL-13	41.6	0	0	0	21.1	0	0	0	0	0	0	0	0	0	0
IIL-15	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
IIL-10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
IIL-79	0	0	100	0	0	0	42	0	0	0	0	0	0	0	0
IIL-19	3.3	0	0	0	1.4	0	0	0	0	0	0	0	0	0	0
IIL-18	0	0	0	0	0	21	0	0	0	0	0	0	0	0	12.5
IIL-22		0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIL-6			0	0	0	0	0	0	0	0	0	0	0	0	0
IIL-80				0	0	0	0	0	0	0	0	0	0	0	0
IIL12					0	0	0	0	0	0	0	0	0	0	0
IIS-15						0	0	0	0	0	0	0	0	0	0
IIL-73.74							0	0	0	0	0	0	0	0	0
IIL-17								0	0	0	0	0	0	0	0
IIL-21									0	0	0	0	0	0	0
IIS-12										100	0	0	0	0	0
IIL-dup.											0	0	0	0	0
IIS49_52												0	0	0	0
IIL57_58													0	0	0
IIL14														0	0
IIL16															0
IIL-68															

Table 2. Geographic areas of distribution and overlap of siblings and cytotypes within the *Simulium arcticum* complex.

	Distribution (km^2)	Number of contacts with siblings	Number of contacts with cytotypes
Sibling			
S. saxosum IIL-2	2,829,500	8	16
S. negativum IL-3.4	2,819,090	8	18
S. arcticum IIL-1	2,770,444	7	7
S. brevicercum (IIL-st/st)	2,312,022	8	18
S. apricarium IIL-7	1,950,914	6	15
S. arcticum s.s. IIL-3	1,683,894	8	14
S. chromatinum IIL-11	812,216	5	3
S. vampirum IIL-8, IIS-10.11	605,393	6	0
S. arcticum IIS-4	15,736	6	0
Cytotype			
IIL-9	80,355	7	11
IIL-13	39,902	6	5
IIL-15	30,369	5	6
IIL-10	25,729	5	4
IIL-79	20,384	6	3
IIL-19	14,432	6	5
IIL-18	8193	5	5
IIL-22	2776	6	3
IIL-6	1197	3	0
IIL-80	1095	6	2
IIL-12	729	1	0
IIS-15	441	5	3
IIL-73.74	81	4	2
IIL-17	79	5	2
IIL-21	79	4	1
IIS-12	79	3	1
IIL-dup.	79	3	1
IIS-49·52	79	3	0
IIL-57·58	79	3	0
IIL-14	79	0	0
IIL-16	79	1	0
IIL-68	8	4	2

abundant at most sites and constitutes 36% of the distributions of all cytotypes. These statistics suggest that IIL-9 might be a real sibling. However, at Rock Creek, Missoula County, the cytotypes IIL-9 and IIL-19 occur in sympatry along with individuals having the autosomal inversions IL-1 and IS-1 in high enough frequency to test for reproductive isolation of the types. Hardy–Weinberg analysis of the distributions of these autosomal polymorphisms indicates random mating and thus no sibling status for either cytotype (Shields et al. 2007).

The cytotype IIL-10 occurs in isolation at Upper Spring Creek, Fergus County, Montana; however, it also occurs at 10 other sites in Montana along with other siblings and cytotypes. It is notable that IIL-10 occurs in a minority with *S. apricarium* only 7 km to the northwest of Upper Spring Creek.

As discussed earlier, to complement our cytogenetic studies, we have undertaken molecular genetic studies of taxa of the *S. arcticum* complex to better understand the evolution of the group. A case in point is the geographic interface of *S. saxosum* and *S. arcticum* s. s. in the western Rocky Mountain region. *Simulium saxosum* and *S. arcticum* s. s. are probably sister taxa and may have diverged about 70,000 years before the present, based on comparisons of 4 mitochondrial genes (Conflitti et al. 2017). The former is distributed on the western coastal plain from Alaska south to central California, while the latter occurs mostly on the eastern slopes of the Rocky Mountains. The 2 taxa meet at the Coeur d'Alene River in northern Idaho, and that population is composed not only of authentic *S. saxosum* and *S. arcticum* s. s. but also of combinational types (Shields and Kratochvil 2011, Shields 2014). We use the term *combinational* because we are unsure whether these types arose there or have come there via secondary contact. Measurements of centromere size and extents

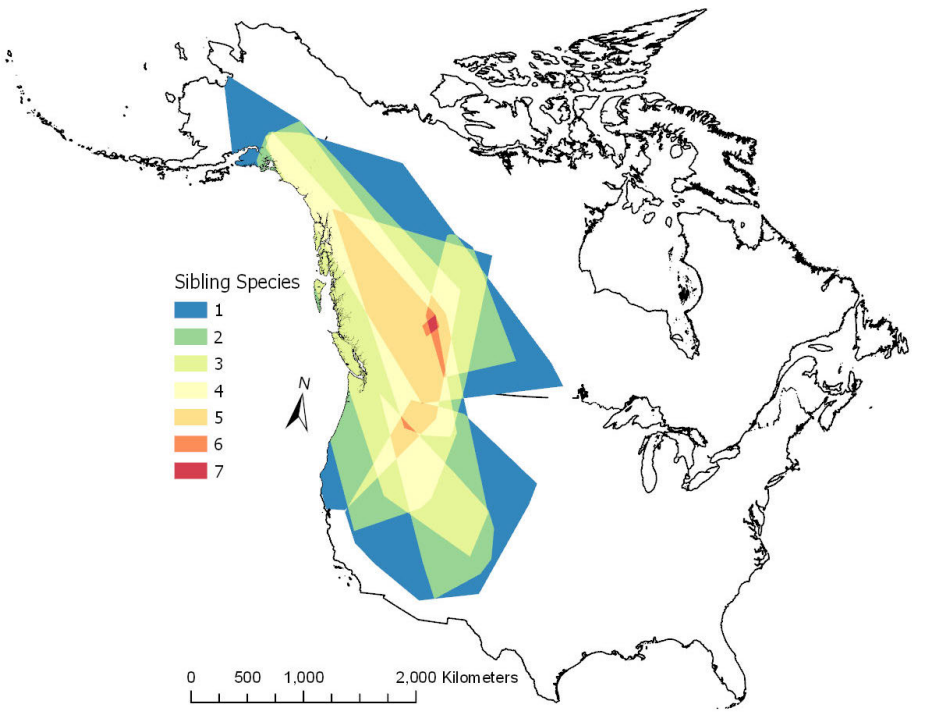

Fig. 2. Distributions of siblings of *Simulium arcticum*, showing overlaps.

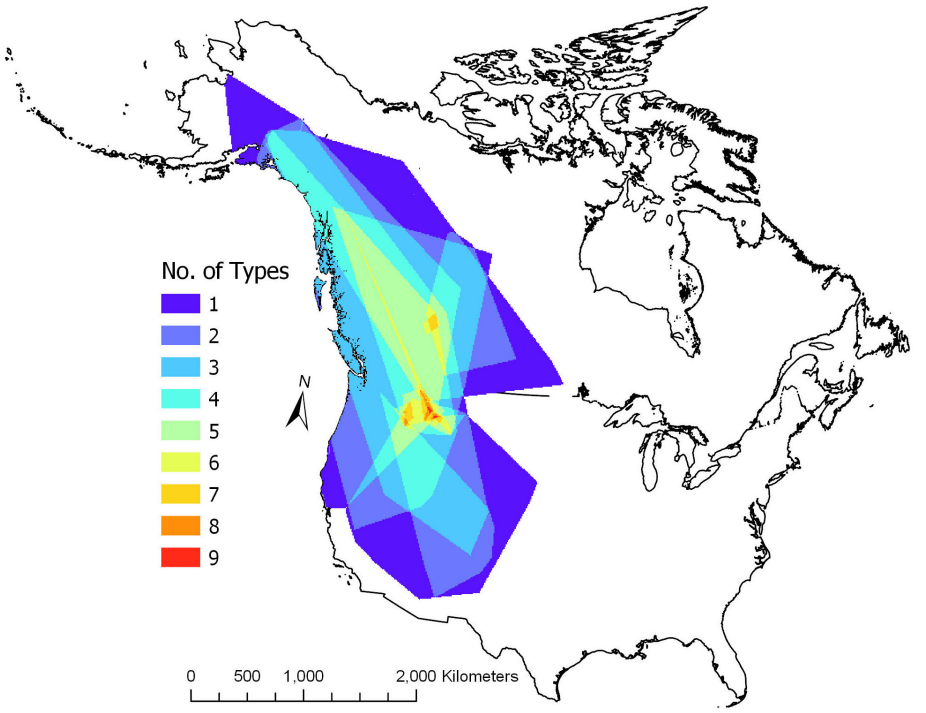

Fig. 3. Distributions of all taxa (siblings and cytotypes) within the *Simulium arcticum* complex, showing overlaps.

TABLE 3. Characteristics of cytotypes within the *Simulium arcticum* complex. n. a. = not available.

Cytotypes	Locations	Number of collections	Proportion of cytotype distribution[a]	Proportion of this type of male present
IIL-9	Bitterroot R. Darby, Bitterroot R. Hamilton, Blackfoot R., Blackfoot R. Sunset Road, Blackfoot R. Roundup, Clark Fork Bearmouth, Clark Fork Bonner, Clark Fork, Turah, Clark Fork Sanders Co., Clearwater R. mouth, Clearwater R. Idaho, Dearborn R. Little Blackfoot, Elliston, Flat Cr. Wash., Rock Cr. Missoula Co., Salmon R. Idaho, Selway R. Idaho, Spokane River, Wash., Kootenai River, Rapid River, Idaho.	24	0.36	Five of 24 collections > 0.70, nine of 24 collections > 0.50
IIL-13	Clearwater Campground, Jocko R., Kootenai R., Rock Cr.	4	0.18	All four collections < 0.060
IIL-15	Big Hole R., Bison Cr., Bitterroot, Darby, Boulder R. High Ore, Deep Cr., Gallatin R., Flint Cr. Hall, Jocko R., Kootenai R., Madison R., Prickly Pear, Ash Grove, Yellowstone Chico, Yellowstone R.	13	0.13	Two of 13 collections > 0.360, all others < 0.140.
IIL-10	Bar-19, Fergus Co., Deep Cr., Lt. Blackfoot R., Elliston, Flint Cr. Hall, N. Fork, Spring Cr., Prickley Pear, Ash Grove, Trout Cr., Upper Spring Cr., Yellowstone River, Chico.	10	0.11	Two of ten were all IIL-10, four of ten were > 0.400
IIL-79	Clearwater R., Idaho, Lt. Salmon R., Lochsa R., Rapid R., Salmon R., Selway R., St. Joe R., Tucannon R.	11	0.09	Four > 0.666, the remainder < 0.321
IIL-19	Bitterroot R., Darby, Bitterroot R., Hamilton, Blackfoot R. Russel Gates, Blackfoot Sunset Hill R., Blackfoot Roundup, Clark Fork, Bonner, Clark Fork, Turah, Clearwater Campground, Clearwater R. mouth, Kootenai R., Rock Cr.	11	0.06	Five > 0.400, six < 0.371.
IIL-18	Bison Cr., Jefferson Co., Boulder R. High Ore, Canyon Cr., Lt. Blackfoot, Elliston, Trout Cr., mouth, Trout Cr. Six m., Wise River.	7	0.04	One > 0.155, six < 0.066
IIL-22	Clearwater Campground, Swan R., Tucannon R., Yaak R.	4	0.01	All four < 0.197
IIL-6	Delta Clearwater R., Monument Cr. Alaska	2	0.005	All males were IIL-6

TABLE 3. Continued

Cytotypes	Locations	Number of collections	Proportion of cytotype distribution[a]	Proportion of this type of male present
IIL-80	Clearwater R., Idaho, Tucannon R., Wash.	5	0.005	One = 0.455, four < 0.190.
IIS-15	Kootenai R., Yaak R., Lincoln Co. Montana	2	0.0004	One = 0.358, the other = 0.067
IIL-12	Smith R., Calif., Rogue and Illinois R. Ore.	2	0.0004	n. a.
IIL-73·74	Big Hole @ Wise R., Wise River.	2	0.0004	One = 0.561, the other = 0.143
IIL-17	Rock Cr. Montana, Selway R., Idaho.	6	0.0003	<0.033
IIL-21	Latah Cr., Wash.,Tucannon R., Wash.	4	0.0003	One = 0.876, the other three < 0.047
IIS-12	Boulder River, Sweet Grass Co.	1	0.0003	0.202
IIL-dup.	Boulder R., Sweet Grass Co., Montana	1	0.0003	0.250
IIS-49·52	Water Cr., Humbolt Co., Nevada	1	0.0003	n. a
IIL-57·58	Metolius River, Oregon	1	0.0003	n. a.
IIL-14	Great Bear River, Nw. Territories	1	0.0003	n. a.
IIL-16	River of Two Mountains, Nw. Territories	1	0.0003	n. a.
IIL-68	Trout Cr., mouth, Lewis and Clark, Co., Montana	5	0.00004	Two > 0.365, three < 0.268

[a]Values indicate the proportion of each cytotypic geographic distribution.

of chromosome pairing suggest that the population at the Coeur d'Alene is the remnant of an ancestral group that gave rise to *S. saxosum* in the west and to *S. arcticum* s. s. in the east (Shields 2014). This scenario may argue for a sympatric origin of the 2 taxa at the Coeur d'Alene River or somewhere nearby. If the 2 taxa came into secondary contact after allopatric speciation, centromere and pairing measurements would have been markedly different (Shields 2014).

All conclusions of this study rest on the assumption that origins of taxa can be deduced from analysis of present-day populations. While it may be impossible to predict origins and modes of differentiation from present distributions, the weight of evidence that nearly all cytotypes share distributions with sibling species suggests the possibility of a sympatric mode of speciation.

ACKNOWLEDGMENTS

GFS was supported in Alaska by a grant from the National Science Foundation Polar Programs (DEB 80-11094) and by the Alaska Council for Science and Technology (F81-16). Carroll College provided salary for research time for GFS through the James J. Manion Endowed Chair of Biological Sciences Fund. The M.J. Murdock Charitable Trust provided grants (2003196 and 2005233) to GFS for student support, equipment, supplies, and travel to collection sites. The National Geographic Society (7212-02) provided support for travel and equipment to GFS. The Clarence A. (Bud) Ryan Cash Grant for Undergraduate Research Fund provided salary for students. The Department of Life and Environmental Sciences at Carroll College provided student salaries, space, equipment, and supplies. The following undergraduate students contributed to this research: Ashley Rhodes, Brian Blackwood, Tonya Santoro, Calli Riggin, Kathryn Styren, Christina Marchion, Tracie Michael, Lindee Strizich, Judi Pickens, Gregory Clausen, Michelle Van Leuvan, Brooke Christiaens, Phil Lenoue, Michael Kratochvil, Amber Hartman, Jeanna Van Hoey, Jessica Brisson, Chance Stewart, Shelby Olson, Morgan Spear, and Taryn Stewart. Pat, John, and Kelly Shields helped with collections. We thank Dr. William S. Procunier for allowing us to include his unpublished data from western Canada. We

thank Drs. Doug Currie and Ida Conflitti for the inclusion of the southwest USA data. We also especially thank Dr. Peter Adler, Department of Entomology, Clemson University, for reviewing an earlier draft of this manuscript, for inclusion of his data on *S. arcticum,* and for his encouragement, continued interest, and support of our work. The late Klaus H. Rothfels is remembered for initially starting our interest in black flies.

Literature Cited

Adler, P.H., and R.W. Crosskey. 2018. World blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomy and geographic inventory [2018]. http://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf

Adler, P.H., D.C. Currie, and D.M. Wood. 2004. The black flies (Simuliidae) of North America. Comstock, Cornell University Press, Ithaca, NY. 941 pp.

Ayala, D., M. Fontaine, A. Cohuet, D. Fontenille, R. Vitalis, and F. Simard. 2011. Chromosomal inversions, natural selection and adaptation in the malaria vector, *Anopheles funestus.* Molecular Biology and Evolution 28:745–758.

Conflitti, I.M., G.F. Shields, R.W. Murphy, and D.C. Currie. 2017. Resolving evolutionary relationships in closely related nonmodel organisms: a case study using chromosomally distinct members of a black fly species complex. Systematic Entomology 42:489–508.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA. 545 pp.

Feder, J.L., and P. Nosil. 2009. Chromosomal inversions and species differences; when are genes affecting adaptive divergence and reproductive isolation expected to reside within inversions. Evolution 63: 3061–3075.

Gavrilets, S. 2014. Models of speciation: where are we now? Journal of Heredity 105:743–755.

Guerrero, R.F., F. Rousset, and M. Kirkpatrick. 2012. Coalescent patterns for chromosomal inversions in divergent populations. Philosophical Transactions of the Royal Society B 367:430–438.

Hoffmann, A.A., and L.H. Rieseberg. 2008. Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation. Annual Review of Ecology, Evolution and Systematics 39:21–42.

Love, R.R., A.M. Steele, M.B. Coulebaly, S.F. Traore, S.J. Emrich, M.C. Fontaine, and N.J. Besansky. 2016. Chromosomal inversions and ecotypic differentiation in *Anopheles gambiae:* the perspective from whole-genome sequencing. Molecular Ecology 25: 5889–5906.

Malloch, J.R. 1914. American black flies or buffalo gnats. United States Department of Agriculture Bureau of Entomology Technical Series, No. 26. Government Printing Office, Washington, DC. 83 pp.

Rothfels, K.H. 1956. Black flies: siblings, sex and species groupings. Journal of Heredity 47:113–122.

Rothfels, K.H. 1979a. Cytotaxonomy of black flies (Simuliidae). Annual Review of Entomology 24:507–539.

Rothfels, K.H. 1979b. Chromosomal variation and speciation in black-flies. Symposium of the Royal Entomological Society of London 10:207–224.

Rothfels, K.H. 1981a. Cytological approaches to the study of blackfly systematics and evolution. Pages 67–83 *in* M.W. Stock, editor, Application of genetics and cytology in insect systematics and evolution. Forest Wildlife and Range Experimental Station, University of Idaho, Moscow, ID.

Rothfels, K.H. 1981b. Cytotaxonomy: principles and their application to some northern species-complexes in *Simulium.* Pages 19–29 *in* M. Laird, editor, Blackflies. Academic Press, New York, NY.

Rothfels, K.H. 1989. Speciation in black flies. Genome 32:500–509.

Rothfels, K.H., R.M. Feraday, and A. Kaneps. 1978. A cytological description of sibling species of *Simulium venustum* and *S. vercundum* with standard maps for the subgenus *Simulium* Davies (Diptera). Canadian Journal of Zoology 56:1110–1128.

Shields, G.F. 2013. The significance of sex-linked chromosomal inversions in the speciation process of the *Simulium arcticum* complex of black flies (Diptera: Simuliidae). Monographs of the Western North American Naturalist 6:64–86.

Shields, G.F. 2014. Incipient speciation and additional diversity within the *Simulium arcticum* complex of black flies (Diptera: Simuliidae). American Midland Naturalist 172:1–13.

Shields, G.F. 2016. Determining causal factors of diversity in the *Simulium arcticum* complex of black flies (Diptera: Simuliidae). Trends in Entomology 12:73–80.

Shields, G.F., and M.J. Kratochvil. 2011. A remnant of an incipient speciation event in the *Simulium arcticum* complex (Diptera: Simuliidae). American Midland Naturalist 166:239–251.

Shields, G.F., J.A. Pickens, G.M. Clausen, and L.M. Strizich. 2007. Reproductive status of cytoforms in a black fly complex in Montana. Intermountain Journal of Science 13:32–43.

Shields, G.F., and W.S. Procunier. 1982. A cytological description of sibling species of *Simulium* (Gnus) *arcticum* (Diptera: Simuliidae). Polar Biology 1: 181–192.

Shields, G.F., and J.P. Shields. 2018. Geographic structure of sibling species and cytotypes of the *Simulium arcticum* complex (Diptera: Simuliidae) in rivers of central Idaho and southeastern Washington, United States of America. Canadian Entomologist 150: 366–377.

Swenson, N.G., and D.T. Howard. 2005. Clustering of contact zones, hybrid zones and phylogenetic breaks in North America. American Naturalist 166:581–591.

Received 18 October 2018
Revised 22 January 2019
Accepted 7 February 2019
Published online 12 July 2019