Differential Development of Male and Female Larvae of a Black Fly Complex (Diptera: Simuliidae)[1]

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Abstract Some adult female black flies (Diptera: Simuliidae) bite humans and can be pests in high numbers. In areas of high black fly abundance, larvicide-based suppression programs reduce the emergence of adults from rivers and streams. However, little is known about whether either of the sexes develops or emerges first. Descriptions of sexual development might help control managers decide when application of pesticides is most effective. For the past 14 yr the cytogenetic diversity within the *Simulium arcticum* (Malloch) complex of black flies in the Pacific Northwest has been studied and described. These descriptions have been accompanied by an "on-slide" identification of the gonads and, thus, the sex of individual larvae. Consequently, a very large data set is available to describe sex ratios during larval development and determine if either sex develops before the other. The sex of >11,000 larvae from 161 collections from 41 geographic locations has been monitored. Larvae having white histoblasts were chosen for chromosome studies, but no selection of either sex was made. Males outnumbered females in first collections at almost all sites and those made before 31 March of any year. Between 31 March and 15 April, females slightly outnumbered males, but after 15 April the ratio of the sexes was similar. These data also suggest that this approach might be inexpensive and useful in the immediate determination of sex ratios because an estimate of the ratios could be made within hours of application of larvicides.

Key Words black flies, larvae, sexual development, larvicide, control

Most female black flies (Diptera: Simuliidae) require a blood meal for egg development, and they can be vectors of blood diseases (Adler et al. 2004). About 13% of North American species of black flies bite humans, and 54 species have been reported to bite humans at least once (Adler et al. 2004). Swarms of biting and nonbiting black flies can interfere with outdoor recreational activities, and from 5 to 10 flies captured with 10 overhead sweeps with an insect net are deemed unacceptable (Gray et al. 1996). Thus, programs to control black flies have been established in areas where numerous flies occur. Black fly eggs, larvae, and pupae reside in streams and rivers, and thus, suppression programs have been directed at actively feeding larvae in aquatic systems because it is difficult to reduce population numbers after the emergence of adults.

Moreover, it is not quantitatively known if either sex develops before the other (Adler et al. 2004). It may be evolutionarily advantageous for males to develop

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slightly earlier than females because many males would then be available to inseminate females when they eventually emerge. It is generally known that males may develop faster and pupate earlier than females that might remain as larvae to acquire more fat reserves for egg development (Adler et al. 2004), but the detail of differential development of the sexes is not quantitatively known. Though the present study describes sex ratios only of members of the *Simulium arcticum* (Malloch) complex, such large and long-term data sets are rare yet could contribute to a better understanding of development in black flies and to effective control treatments.

Knowledge of sex ratios during periods of larval development could aid control programs with respect to timely applications of larvicides. For example, if male black fly larvae develop sooner than females this knowledge could influence the timing of treatment decisions (e.g., leave male larvae in the system and treat just before females eventually emerge [J. Walz, pers. comm.]).

There are 24 active black fly control programs in 17 states and 4 Canadian provinces that conduct larvicide applications in streams and rivers to reduce the emergence of black flies (Adler et al. 2004; E. Gray, pers. comm.). These programs are expensive. For example, the yearly budget of a large control program in Minnesota is estimated to be $300,000.00 (J. Walz, pers. comm.). A recent estimate indicates that 2,300 L of Vectobac 12AS (currently the preferred treatment) valued at $9.00/L were used in a single treatment session on the Colorado River to suppress the emergence of black flies (E. Gray, pers. comm.). Thus, it might be informative to control programs to know if the larvicide being used is directed at females that reproduce and potentially bite as adults.

Direct assessment of differential sex ratios at emergence might be obtained through emergence traps (Davies 1950), but this method is expensive, labor intensive, and usually conducted only in a restricted area. Alternatively, indirect assessment of differences in rates of development of each sex could be obtained by monitoring larval development via collections of larvae intended for other purposes as in the present study.

Many taxa of black flies exist as complexes of sibling species solely differentiated by the linkage to maleness of chromosomal inversions in larval salivary gland chromosomes (Adler et al. 2004; Rothfels 1979, 1989; Rothfels and Featherston 1981; Shields 2013, 2014a, 2014b; Shields and Kratochvil 2011; Shields and Procunier 1982; Shields et al. 2009). For the past 14 yr, I have studied the genetic diversity within the *S. arcticum* complex in the northwestern region of the United States through analysis of polytene chromosomes (Shields 2013). Because linkage to maleness of chromosomal inversions appears to be a paradigm of this process, all of my cytogenetic analyses are accompanied by an "on slide" determiner of sex in the form of the larval gonad. Consequently, identifications of sex for $>$16,000 larvae of the *S. arcticum* complex from 241 collections at 67 geographic locations are available and these collections can be used to indirectly assess development of both sexes.

Because male larvae are usually the "informative" sex with regard to cytotype, cytogeneticists plan collections to correspond with the peaks of male larval development (Shields 2013). For example, at Rock Creek in western Montana in each of 4 d during the peak of larval development, males outnumbered females by 3:2 (Shields et al. 2007). Thus, in my experience male larvae usually outnumber females at this time, but this process has not been quantitatively documented across a species complex. Herein are reported the results of these analyses.

Materials and Methods

More than 11,000 larvae of the *S. arctium* complex of black flies from 161 collections taken at 41 geographic locations from freshwater streams in the states of Montana, Idaho, and Washington (Fig. 1) were analyzed to determine the sequence of development of male and female larvae. These data emanated from an earlier study that determined the cytogenetic diversity within the *S. arcticum* complex through comparisons of giant polytene chromosomes of the salivary glands of each larva (Shields 2013). Because healthy larvae, decreased parasitic loads, and chromosomes of analyzable quality occur in spring (Shields 2013), most larvae analyzed here were collected from early March through late May. My collection efforts for cytogenetic analysis were directed at obtaining about equal numbers of male and female larvae at the penultimate (white to gray histoblast) stage of development because those larvae generally render chromosomes of excellent quality. The sex ratio of females to males for each collection was determined regardless of the year they were collected. For each site, Global Positioning System (GPS) coordinates were reported, collection dates were ordered from earliest to latest, and the ratio of females to males was determined.

All larvae were fixed at each site in fresh, cold Carnoy's fixative (Parker 1964), which was changed until it became clear (usually four changes) (Shields and Procunier 1982). Larvae were sorted to the *S. arcticum* complex based on the morphology of gill histoblasts, postgenal clefts, and dorsal head patterns (Currie 1986), were opened ventrally to expose gonads and polytene chromosomes, and stained in Feulgen (Rothfels and Dunbar 1953). Sexes of all larvae were determined by the presence of either ovaries or testes.

Three separate analyses were conducted. First, sex ratios were determined for the first collections made at all sites. Secondly, the sex ratios of larvae collected before and after 31 March were determined. In a separate analysis, sex ratios were determined for three time intervals—early (before 31 March), middle (between 31 March and 15 April), and late (after 15 April). F statistics, degrees of freedom, probability values, means, and standard errors were determined for each category (Zar 1984).

Results

The sex ratios of 11,239 larvae from 161 collections at 41 geographic locations were determined (data available from author and Fig. 1). Among the first collections made at the 41 sites, males outnumbered females at 34 sites. Females outnumbered males at six sites, and one site had equal numbers of each sex (data available from author). Males were present in larger numbers than females at most sites in early spring. In the initial comparison, the mean ($\pm$ SE) proportion of the larvae collected before 31 March that was female was 0.3791 ± 0.0181 (Fig. 2). This proportion was significantly higher ($F = 23.72$; df $= 1$; $P < 0.001$) after 31 March, with a mean of 0.5114 ± 0.0188. Similarly, analysis of those proportions

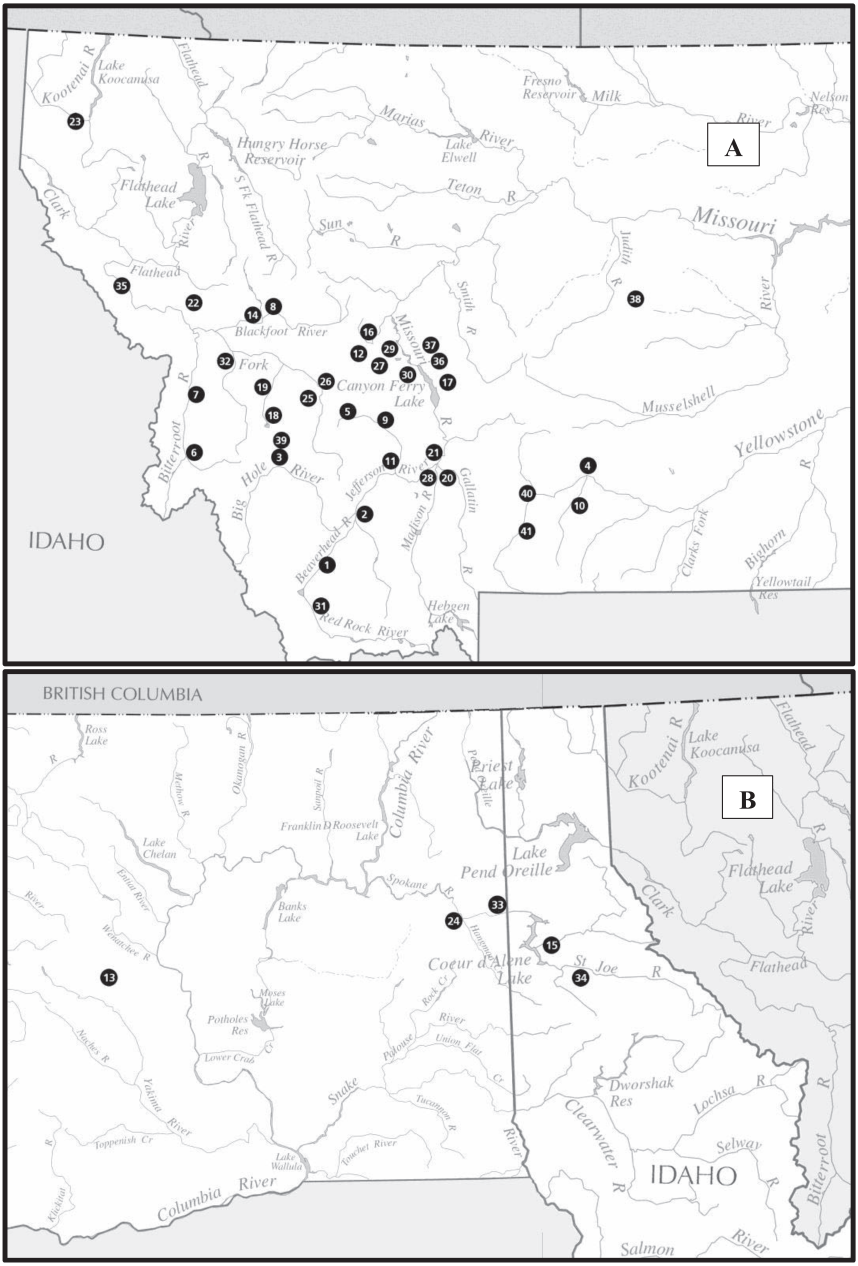

Fig. 1. Geographic locations in (A) Montana and (B) Idaho and Washington of collections of *Simulium arcticum* larvae analyzed in this study. Numbers inside circles correspond to locations alphabetically listed raw data, available from the author upon request.

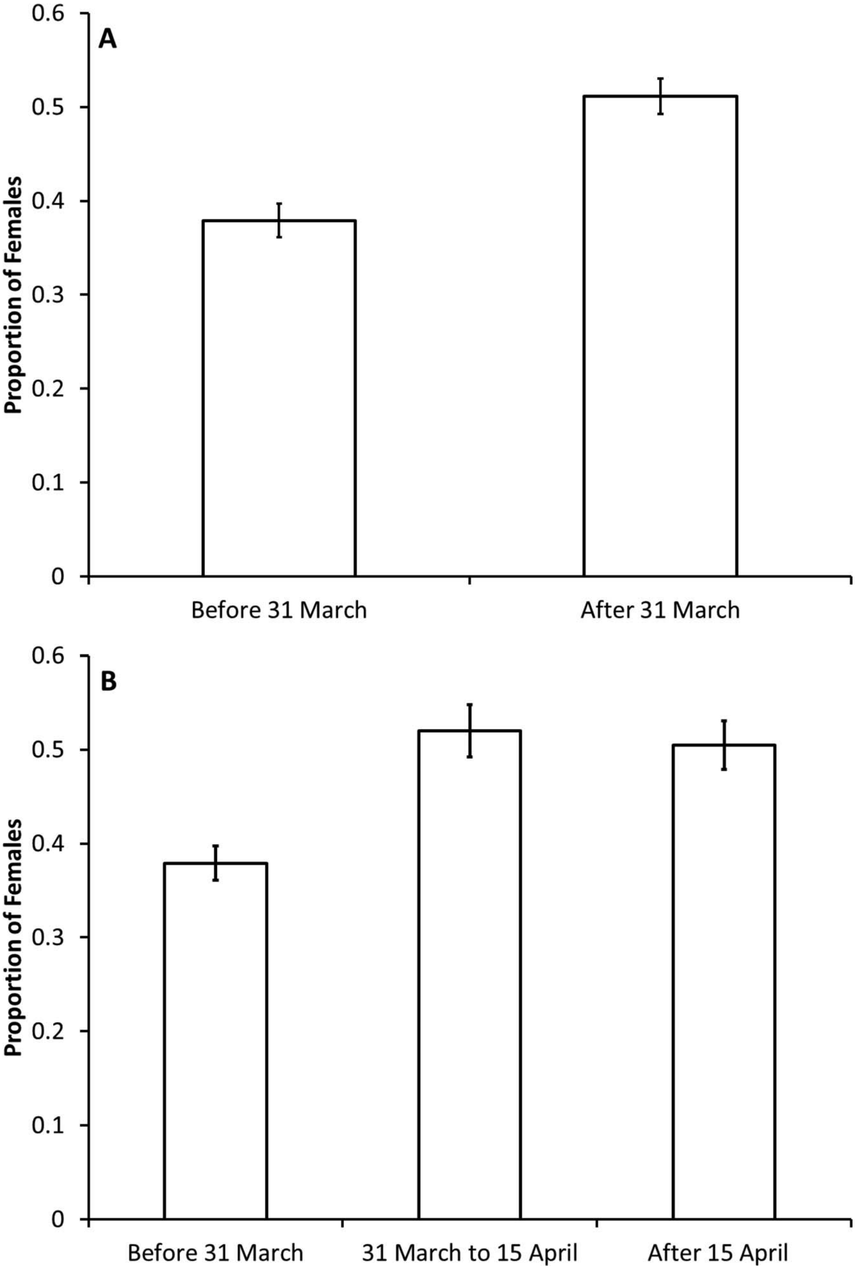

Fig. 2. (A) Proportions of female larvae in total numbers of *Simulium arcticum* collected before 31 March and after 31 March and (B) proportions of female larvae in total numbers of *S. arcticum* collected in the intervals prior to 31 March, between 31 March and 15 April, and after 15 April.

relative to the intervals of time showed that males outnumbered females in collections made before 31 March, but females outnumbered males during the interval of 31 March to 15 April, while the proportions were essentially equal in the time period after 15 April (Fig. 2). Mean proportions of females from those intervals were 0.3791 $\pm$ 0.0181, 0.5198 $\pm$ 0.0277, and 0.5047 $\pm$ 0.0257, respectively (Fig. 2).

Discussion

Until this analysis, no long-term quantitative assessment of development rates of male and female black fly larvae within an entire species complex had been made. My results suggest that for the *S. arcticum* complex, males outnumber females at the penultimate histoblast stage of larval development in collections before 31 March. Presumably, these males then emerge before females. As stated earlier, it may be advantageous for male larvae to develop and emerge first because many of them would be available to inseminate females when those females emerge. The opposite scenario, in which females would emerge first seems maladaptive because no or few males would be available to inseminate those emerging females. I am aware that these data suggest only an earlier development for males; they do not indicate an earlier emergence for either sex. However, it is unlikely that females would emerge first because they may require more time in the larval stage to acquire nutrients for egg development (Adler et al. 2004). Determination of actual sex ratios at emergence would require emergence traps, which were not used in this study.

Though these results suggest an earlier development of males to the penultimate histoblast stage, they do not indicate how much earlier than females males might emerge. Nonetheless, these observations may be informative in understanding the biology of black flies in general as well as being helpful to managers of control programs. While a later development of female larvae applies only to the *S. arcticum* complex analyzed here, from a control standpoint it may be important to treat freshwater streams late in the spring when female larvae are proportionately more abundant. This approach to determination of sex ratios in developing larvae might be practical in other complexes because many hundreds of larvae could be fixed, stained, and sexed very quickly. As stated earlier, this increased level of efficiency may also help suppression programs comply with new regulations set forth by recent changes to the Clean Water Act and the National Pollution Discharge Elimination System.

This analysis would have been biased had the larvae been collected later in spring when females tend to emerge. This might not be a concern in the present study because the number of collections for each of the three temporal categories was similar (i.e., before 31 March = 63, between 31 March and 15 April = 42, and after 15 April = 56).

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