Zootaxa 214: 1-11 (2003)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)
214

A new species of *Stegopterna* Enderlein, and its relationship to the allotriploid species *St. mutata* (Malloch, 1914) (Diptera: Simuliidae)

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Abstract

A new species of black fly, *Stegopterna diplomutata* **n. sp**. (Diptera: Simuliidae) is described and illustrated. This species is most closely related to *Stegopterna mutata* (Malloch, 1914), with which it has long been confused. The two species are most easily distinguished based on the presence or absence of males; *St. diplomutata* is a diploid bisexual species that possesses males, whereas *St. mutata* is a triploid parthenogenetic species that lacks males. The two species are otherwise not distinguishable except through examination of their larval polytene chromosomes. Observations are provided about the evolution of triploidy in *Stegopterna*.

Key words: Simuliidae, *Stegopterna*, cytology, triploidy, evolution

Introduction

Stegopterna Enderlein is a relatively small genus of black flies with 9 nominal species distributed throughout the Holarctic Region. Females are unusual among the '*Cnephia*-grade' simuliines in possessing a simple (as opposed to a bifid) tarsal claw and the presence of exceptionally long hind tibial spurs. The tibial spurs are further distinguished in being distinctly bicolored — the basal three-quarters being brown and the apex contrastingly pale, almost colorless.

The immature stages of *Stegopterna* typically occur in small, temporary- or semi-permanent woodland streams. Larvae attach themselves to a variety of submerged substrata and are relatively easy to observe and collect. Pupae, on the other hand, are exceedingly cryptic and often difficult to find, occurring typically on the undersurfaces of stones or deep within the streambed.

Accepted: 9 June 2003; published: 18 June 2003

Although the monophyly of *Stegopterna* has not been seriously challenged, the genus has never been subjected to a taxonomic revision of included species. Until very recently, only two nominal species were recognized from the Nearctic Region: *St. mutata* (Malloch, 1914) and *St. emergens* (Stone, 1952) (Crosskey and Howard 1997). However, cytological studies have revealed that *mutata* of authors actually consisted of 5 or more reproductively isolated sibling species (Basrur and Rothfels 1959, Madahar 1968). The name *permutata* (Dyar & Shannon, 1927) – long considered a junior synonym of *mutata* Malloch – was recently associated with "cytospecies X" of Madahar (1968) (Currie 1997). Currie (loc. cit.) also recorded for the first time the occurrence of *St. decafilis* Rubtsov, 1971, in North America. This brought to four the number of named *Stegopterna* species recorded from the Nearctic Region. In this paper we formally describe a fifth species, and provide notes about the origin of triploidy in *Stegopterna*.

Materials and methods

Larvae for chromosomal analyses were collected from Booth's Rock Trail Trickle (45°30'N 78°23'W) and Costello Creek (45°36'N 78°21'W), Algonquin Provincial Park, Ontario, Canada, at weekly intervals from April 24 1989 to July 24 1989 (calendar weeks 17 to 30). Material was collected into Carnoy's fixative (1 part glacial acetic acid: 3 parts absolute ethanol). Larval salivary gland polytene chromosomes were prepared according to the methods of Rothfels and Dunbar (1953). By convention, fixed inversions are underlined (e.g. IS-1 which distinguishes *St. emergens* from *St. mutata* and *St. diplomutata*) but floating inversions (chromosomal polymorphisms) are not. Thus IS-1 in *St. diplomutata* is a floating inversion with breakpoints different from those of the fixed inversion, IS-1. Floating inversions are named in the order that they were discovered; thus, IL-5 was the fifth inversion found in *St. diplomutata* and IL-6 was the sixth.

Stegopterna diplomutata **Currie & Hunter n.sp.**

Cnephia mutata: of authors, not Malloch, 1914 (numerous references to diploid populations of the *St. mutata* complex)
Cnephia mutata '2n': Basrur 1957: 4-28
Cnephia mutata 'diploid form' Basrur & Rothfels 1959: 571-589
Stegopterna mutata: of authors, not Malloch, 1914 (numerous references to diploid populations of the *St. mutata* complex)

The name *St. diplomutata* is here associated with diploid (i.e., bisexual) populations of the *St. mutata* complex because triploid (i.e., parthenogenetic) populations are much more common of the two species in eastern North America. Accordingly, the name *mutata* probably applies validly to Malloch's (1914) type for that species – a female from Glassboro, New Jersey.

MALE

Wing length ca. 2.5 mm. Scutum velvety brown. All hairs golden. Legs brown; calcipala large, lamellate, more than half as wide as apex of basitarsus. Terminalia (Fig. 1): gonostylus not markedly tapered, bearing two medially directed spinules; ventral plate in ventral view moderately tapered anteriorly; paramere elongate, without apical spines (although closely associated with those adorning the aedeagal membrane); median sclerite with apex bifurcate.

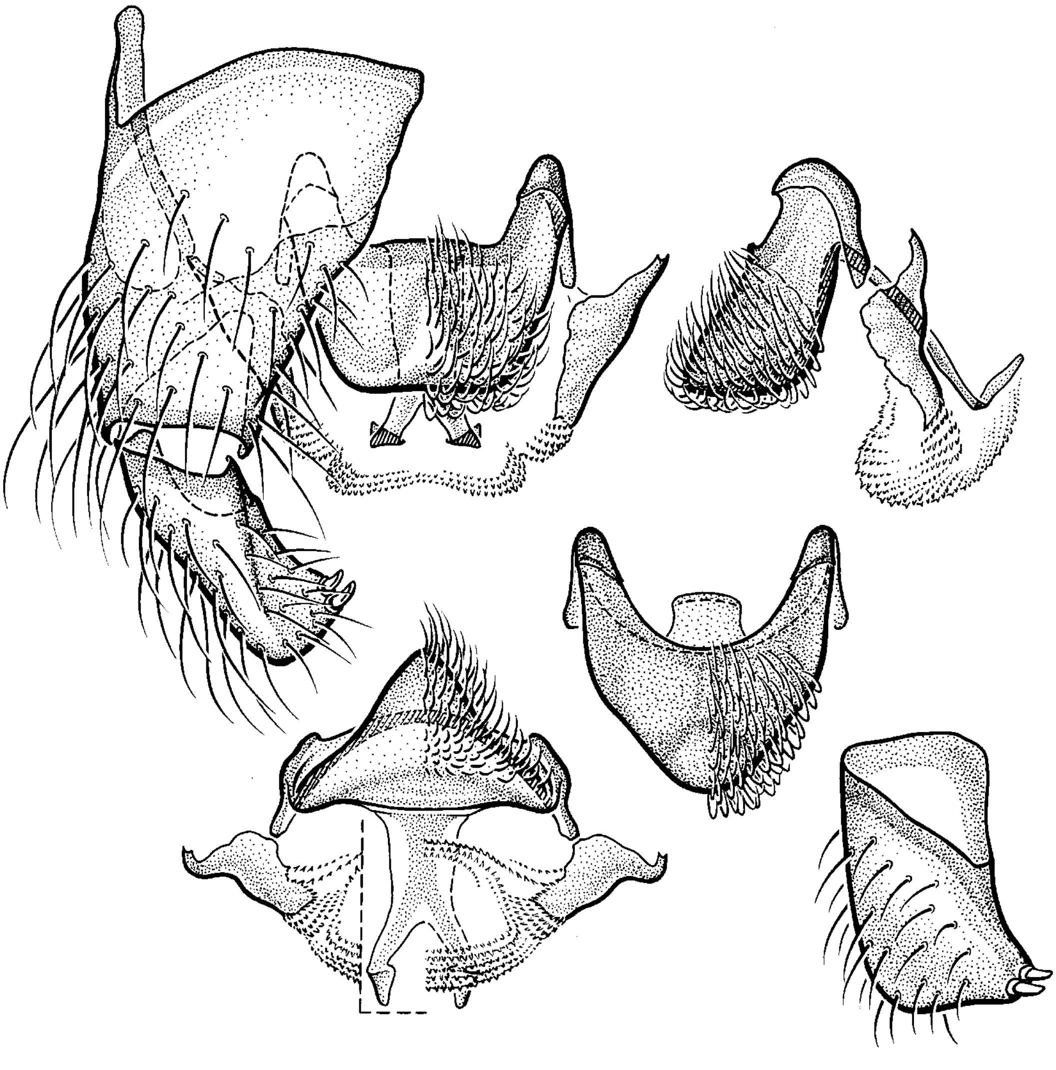


FIGURE 1. Male terminalia of *Stegopterna diplomutata* **n. sp**.: a, ventral view; b, lateral view of ventral plate, median sclerite, paramere, and aedeagal membrane; c, terminal view of same structures; d, inner (dorsal) view of gonostylus; e, alternate view of ventral plate showing shape of posterior margin.

FEMALE

Wing length ca. 3 mm. Scutum dark brown. All hairs golden. Mouthparts with mandibles serrated; lacinia with retrorse teeth; sensory vesicle about one-third as long as palpomere III. Legs brown; calcipala large, lamellate, more than half as wide as apex of basitarsus. Terminalia: anal lobe in lateral view with anterior margin scarcely sclerotized; genital fork with arms expanded into a large, subrectangular, lateral plate. Spermatheca almost spherical with polygonal pattern.

PUPA (Fig. 2).

Length ca. 4 mm. Gill of 12 fine filaments arranged in two vertically divergent groups; a dorsal group of 7 filaments branching (1 + 2) (2 + 2), and a ventral group of 5 filaments branching (1 + 2) (1 + 1). Head and thorax with numerous rounded granules; trichomes simple. Cocoon a shapeless sac covering most of the body.

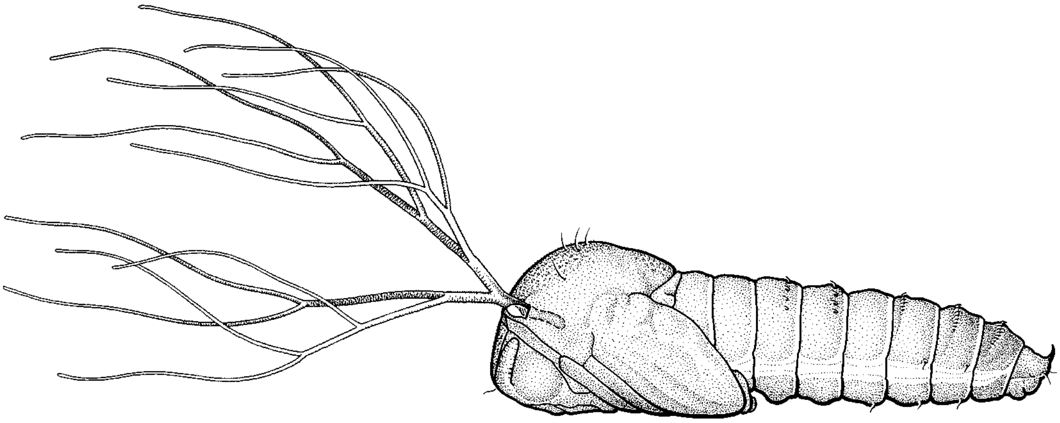


FIGURE 2. Pupa of *Stegopterna diplomutata* **n. sp**. in lateral view with cocoon removed.

LARVA

Length ca. 6 – 7 mm. Body color grayish brown. Head capsule brownish yellow with contrastingly dark headspots. Antenna extended anteriorly well beyond apex of labral fan stalk. Hypostoma with teeth arranged in 3 prominent groups consisting of an elongate median tooth and two lateral lobes. Postgenal cleft inverted v-shaped, extending anteriorly as far as one-third the distance to hypostomal groove. Rectal papillae of 3 simple lobes.

TYPES

The type series was selected from a single locality because two or more species may be included under this name, which is here applied to all diploid populations of the *St. mutata* complex in eastern North America.

HOLOTYPE: Male, frozen dried, double mounted with minuten pinned through dorsum of scutum, and with pupal exuvia in glycerin pinned beneath in microvial. CANADA: Ontario, Nipissing District, Algonquin Provincial Park, Booth Lake, Booth's Rock Trail, 18 May 1992, F.F. Hunter.

The holotype and some larval paratypes are deposited in the Canadian National Collection of Insects, Ottawa. Additional paratypes are deposited in the Royal Ontario Museum, Toronto, the National Museum of Natural History, Smithsonian Institution, Washington, DC, and the Clemson University Arthropod Collection, Clemson, SC.

DERIVATION OF SPECIFIC NAME

The specific name is derived from the combination of “diplo” (a reference to the diploid configuration of the chromosomes) and “mutata”, the name of the closely related triploid species.

DIAGNOSIS

Stegopterna diplomutata is morphologically indistinguishable from *St. mutata* except for the presence of males in the former species. The male and female of *St. diplomutata* are easily distinguished from those of *St. emergens* and *St. decafilis* on the basis of their relatively large calcipala (half or more as wide as apex of basitarsus versus markedly less than half that width in the other two species). Furthermore, the lacinia and mandible of female *St. diplomutata* are armed with retrorse teeth and serrations, respectively. Such armature is lacking from the laciniae and mandibles of *St. emergens* and *St. decafilis*. Males and females of *St. diplomutata* are structurally similar to those of *St. permutata* and the other currently recognized cytotypes of *Stegopterna* from North America (viz., cytotypes 'O', 'W', and 'Y' of Madahar (1969)). However, *St. diplomutata* is distributed from the Great Plains eastward, whereas *St. permutata* and the informally named cytotypes occur west of the Great Plains.

Chromosomally, *St. diplomutata* and *St. mutata* differ from other members of the genus *Stegopterna* (e.g., *St. emergens*) by not having the fixed inversion IS-1 described by Madahar (1969). The IS-1 inversion has breakpoints (relative to the centromere) in the proximal region of section 3 and the distal region of section 6. Furthermore, *St. mutata* and *St. diplomutata* have the nucleolar organizer (NO) in the base of IL as compared with other species in genus *Stegopterna* which have the NO in the base of IS (Madahar 1969).

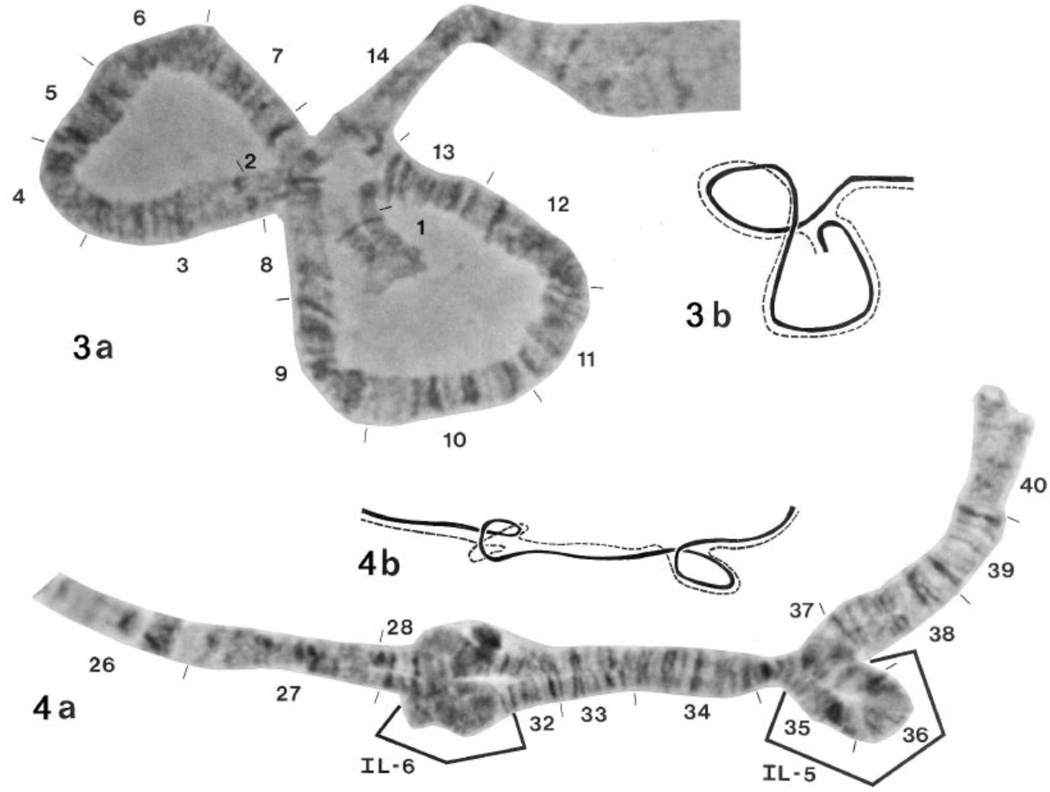
Stegopterna diplomutata differs from *St. mutata* in the following features (Figs. 3, 4, and 5): *St. diplomutata* is diploid and *St. mutata* is triploid. The floating inversions IL-1, IL-2, IL-3, IL-4, IIL-1 and IIL-2 have been found in *St. mutata* (see Basrur and Rothfels, 1959), whereas floating inversions IS-1, IL-5, IL-6 and IIL-2 have been found in *St. diplomutata*. It is conceivable that additional floating inversions may be found in other as-yet undiscovered populations of both species.

DISTRIBUTION

Stegopterna diplomutata is distributed widely in eastern North America, from central Ontario and Quebec south to Alabama.

BIOLOGY

The immature stages of *St. diplomutata* occur typically in small sized, low productivity, temporary- or permanent streams. Aspects of population dynamics have been discussed previously by Davies (1950), Basrur and Rothfels (1959), Davies et al. (1962), Back and Harper (1979), and Adler and Kim (1986). In northern Ontario, *St. diplomutata* is among the earliest developing simuliids in the streams they inhabit (Fig. 6). By the time larvae in the *Simulium venustum/verecundum* complex appear, *St. diplomutata* larvae have largely been replaced by those of the parthenogenetic *St. mutata* triploids.



FIGURES 3-4. a, Chromosome photomicrographs of *Stegopterna diplomutata;* b, diagrams showing pairing of homologues. Fig. 3, IS, Short arm of Chromosome I showing a heterozygote for the IS-1 floating inversion; Fig. 4, IL, Long arm of Chromosome I showing a double heterozygote for the IL-5 and IL-6 floating inversions.

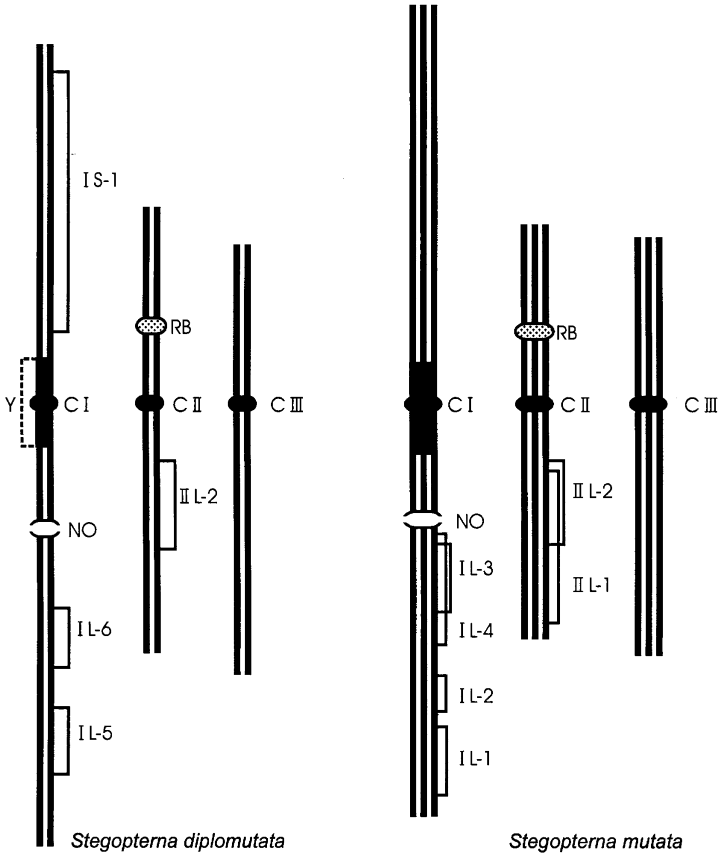


FIGURE 5. Idiograms of *Stegopterna diplomutata* and *St. mutata*. The location of major landmarks are shown: C, centromere; NO, nucleolar organizer; RB, Ring of Balbiani. Brackets to the right of the chromosome arms denote floating inversions (polymorphisms) found in the two species. Note that the breakpoints of IIL-2 are identical in the two species. The bracket to the left of chromosome I in *St. diplomutata* indicates the region that is unpaired in males (the "Y" chromosome) but paired in females.

Cytology of Ontario populations of *St. diplomutata*

At both Booth's Rock Trail and Costello Creek *St. diplomutata* males are the heterogametic sex, with males having an unpaired region at the centromere of chromosome I (CI unpaired). We recognize two cytotypes of *St. diplomutata*.

Cytotype A is homozygous in all arms; there are no floating inversions. Samples at Costello Creek fit this profile. At this site *St. diplomutata* A larvae appear before *St. mutata* larvae and overlap temporally with *Prosimulium fuscum/mixtum* larvae (Fig. 6, Costello Creek).

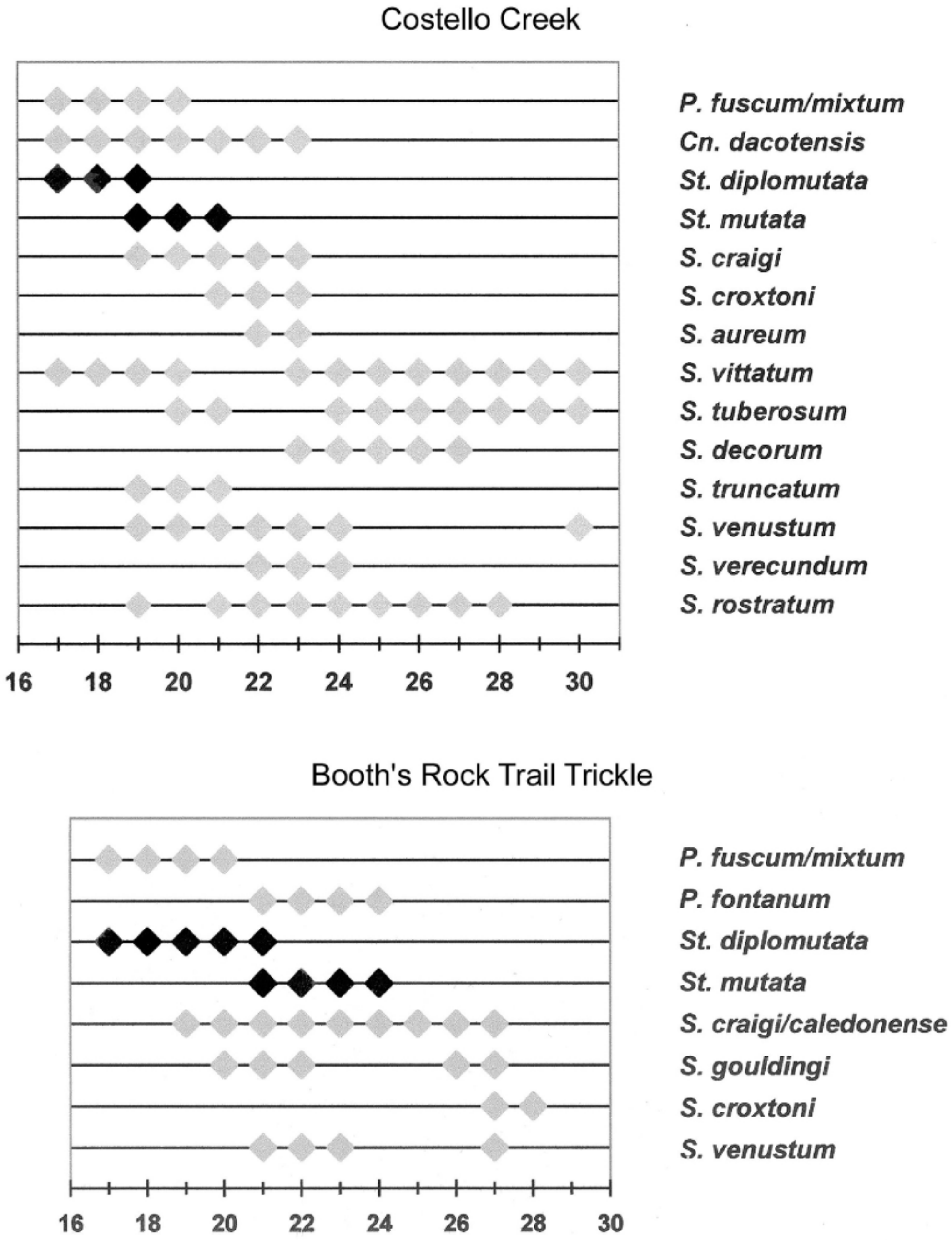


FIGURE 6. Temporal succession of mature black fly larvae found at two different collection sites in Algonquin Provincial Park: Costello Creek, above and Booth’s Rock Trail Trickle, below. Along the x-axis, calendar week is given. Species are from four different genera: P., *Prosimulium*; Cn., *Cnephia*; St., *Stegopterna*; S., *Simulium*.

Cytotype B has been found only at Booth's Rock Trail Trickle. It, too, overlaps temporally with *Prosimulium fuscum/mixtum* and occurs before *St. mutata* (Fig. 6, Booth's Rock Trail Trickle). This cytotype is polymorphic. The IIL-2 inversion of *St. mutata* is found in *St. diplomutata*. Furthermore, there are three novel inversions not hitherto seen in any diploid or triploid population. These are: IS-1, a large inversion occupying almost the entire short arm of chromosome I, with approximate breakpoints between sections 1 / 2 and 13 / 14 (Figs. 3a, 3b); IL-5, a small subterminal inversion in the long arm of chromosome I, with approximate breakpoints 35p (proximal region of section 35) to 37p (proximal region of section 35) (Figs. 4a, 4b); IL-6, a small inversion in the long arm of chromosome I, with breakpoints 28p (proximal region of section 28) to 32p (proximal region of section 32) (Figs. 4a, 4b). Of 10 females and 5 males scored from a Booth's Rock Trail Trickle collection of *St. diplomutata* made on April 25 1989, all males had the CI unpaired (Fig. 5, section indicated by "Y") and all females had a paired CI. The IIL-2 inversion, first found in *St. mutata*, was found in 2 individuals; the novel IS-1 inversion was found in 2 individuals and the novel IL-5 and IL-6 inversions were each found in 6 individuals.

Evolution of triploidy in *Stegopterna*

The *Stegopterna mutata* complex has long been of interest to cytologists due to the existence of chromosomally monomorphic, sexually reproducing, diploid populations and chromosomally polymorphic, parthenogenetic, triploid populations (Basrur and Rothfels 1959). Basrur and Rothfels (loc. cit.) argued that the two do not interbreed, and thus, represent reproductively isolated, distinct biological species. Furthermore, they discuss two potential hypotheses for the evolution of parthenogenetic triploidy in *St. mutata*: (1) triploids are allopolyploids of "polyphyletic" origin and are perpetuated by ameiotic (apomictic) parthenogenesis, and (2) triploids are autopolyploid, not necessarily "polyphyletic" in origin and were perpetuated (at least originally) by meiotic (automictic) parthenogenesis. Against the first hypothesis, they argued that a single population of 3*n* *St. mutata* from Inglewood, Ontario, with four known inversions (IIL-1, IL-1, IL-2, IL-3) associating in 15 different combinations, would require the independent derivation of 15 different triploid clones from a heterozygous diploid progenitor population. The alternative, that all of the Inglewood lines could be derived by segregation from a single progenitor triploid individual (heterozygous for IIL-1, IL-1, IL-2, and IL-3) if parthenogenesis were meiotic, is the more parsimonious explanation and would support the second hypothesis.

Unfortunately, it has not been possible to directly study meiosis in the triploid females, although a proof of meiotic parthenogenesis would be if the progeny of a single polymorphic parthenogenetic female could be scored for inversion polymorphisms and found to be of several different recombinant types.

The widespread existence of a structurally homozygous bisexual diploid form of *St. diplomutata* often co-occurring with the highly polymorphic parthenogenetic triploid form, might suggest a multiple and allopolyploid origin of the triploid; in this case, the homozygous bisexual diploid would contribute the standard sequences for all chromosome arms and the various inverted sequences would have to have been contributed by a closely related hypothetical diploid apomict (i.e., a diploid parthenogenetic form). Although it would not be easy to detect facultative diploid apomicts, such a situation is highly unlikely (Basrur and Rothfels 1959) since it is hard to envision a diploid apomict crossing with a bisexual diploid to form a viable triploid automict.

The critical missing evidence for hypothesis (1) would be the discovery of a new member of the complex that is diploid, parthenogenetic and carries the IIL-1, IL-1, IL-2, and IL-3 inversions whereas the critical missing evidence for hypothesis (2) would be the discovery of a new member of the complex that is diploid, bisexual and carries the IIL-1, IL-1, IL-2, and IL-3 inversions. The discovery of cytotype B of *St. diplomutata* is strong evidence in favor of the feasibility of hypothesis (2).

Thelytoky is when males are totally absent (or very rare and non-functional in a genetic sense). There are two types of thelytoky, namely automictic or meiotic thelytoky when meiosis takes place and apomictic or ameiotic thelytoky when only mitotic-type divisions take place. White (1973) considers it undesirable to group thelytokous and bisexual biotypes together under the same species-name. We are in agreement, especially since morphological traits are evident to separate the thelytokous triploid and the bisexual diploid populations from one another.

Acknowledgments

This research was supported, in part, by NSERC Discovery Grants to DCC and FFH. We thank Peter H. Adler, Clemson University, for his comments on the manuscript. Ralph Idema rendered Figures 1 and 2, and Patrice Stevens-Bourgeault assisted in the preparation of Figures 3 to 6.

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