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Genomic changes in speciation of the family Chironomidae, Diptera

ABSTRACT

The mode of speciation adopted by a species or group of related species of animals (insects) is clearly determined to a certain extent by the architecture of their genetic system. Chromosome rearrangements, the localization and appearance of the heterochromatin as well as the reproductive relations in sympatric, allopathric speciation and introgression process of different species of Chironomidae family are considered. A high chromosome polymorphism and changes in the constitutive heterochromatin are shown as predominant mechanism in the phyletic evolution of some species. Based on an analysis of inversions in different chromosome arms, a chromosome evolution in the "plumosus" group (genus *Chironomus*) is traced. It was found that the floating inversions in an initial standard karyotype became fixed in a derived karyotype and underlined that the heterochromatin is a dynamic element in the speciation of family Chironomidae. In addition, hybridization tests revealed that pre - and postmating isolating mechanisms were operating in process of formation in different sibling species. It is concluded that the species with overlapping ranges are characterized by more complex chromosome changes, while in species with allopatric speciation there are less chromosome rearrangements. In this case, the distance between the populations is a very important factor as well.

Key words: Chironomidae, phyletic evolution**,** sympatric speciation, allopatric speciation, introgression

Introduction

Speciation is ultimately an adaptive process that involves establishment of intrinsic barriers to gene flow between closely related populations by development of reproductive isolating mechanisms. A study of speciation is, to a considerable extent, a study of the genetics and evolution of premating and postmating reproductive isolating mechanisms (Bush, 1975; Keyl, 1962; King, 1993; Kiknadze et al., 2008). In the development of these mechanisms, gene flow is restricted or completely suppressed, thus resulting in the accumulation of gene differences and allowing evolutionary divergence of the two resulting genomes. Speciation may occur either by spatial separation of populations (allopatric speciation) or within the population (sympatric speciation). In sympatric speciation a premating reproductive isolating mechanism may arise before a population shifts to a new niche (Bush, 1975). Speciation is therefore related to the biological features of a group of specimens in the population: for instance in their ability to adapt to a certain area or niche in a biotope.

For the family Chironomidae there are no data available on reproductive isolating mechanisms within and distant populations or on the ways in which they might develop. The Chironomidae demonstrate an unusually wide range of ecological adaptations and many species are found in different kinds of water basins. For this reason, studies on the Chironomidae could play an important role in establishing some general principles of speciation. The purpose of this review is to explore the reproductive relationships in model species of the family Chironomidae and to investigate to what extent and under what circumstances genome changes may initiate speciation process.

Two model groups of family Chironomidae are used for tracing the path of speciation: first, the sibling species of the plumosus group of the genus *Chironomus* Meigen which are

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RESEARCH ARTICLE

symbiotopic, and second, the sibling species of genus *Glyptotendipes* Kieffer which are allobiotopic. The path of speciation in homosequental, in closely related and separating populations of different species will be followed. The methodology includes external morphology of different metamorphic stages, cytogenetic and molecular analysis (FISH method) (Michailova et al., 2009; Ilkova et al., 2013) and degree of hybridization. (Michailova, 1994a).

First will be considered the phyletic evolution, which is related to gradual changes in the karyotype enhancing the adaptive potential of the species and resulting in the microevolutionary differentiation of populations. Good example is *Chironomus plumosus* L. which katyotype and external morphology of larvae and imagos (Figure 1a) showed changes in its Palaearctic range (Michailova & Fischer, 1986). In a number of European populations (Switzerland, Finland, Hungary, Bulgaria, Russia) the species is not considered to be polytypic (Maximova, 1976) but as highly polymorphous, characterized by the presence of a polymorphous system of inversions which involved the transition of a standard homozygous type of the karyotype through heterozygotization into another homozygous type (Michailova & Petrova, 1991, Butler et al., 1999; Michailova et al., 2008). Tracing a chromosome polymorphism in different Palaearctic populations of *C. plumosus* a specially built up mechanism was found manifested by the formation of this polymorphous system. In all populations it affected the chromosomes AB and CD. So, inversions building up the polymorphous system, realized by inversions, are common for geographically distant populations. However, in different populations these common inversions of which the polymorphous system is constricted showed some fluctuations. For instance, the homozygous inversions in arm A have priority in populations from Hungary and Switzerland (Neuchatel) and the Russia (Chernobyl). Therefore, inversions have varying selective priority in different ecologic-climatic conditions. In all investigated populations there is a variation in the frequency of aberrations depending on the specific conditions of the habitat. The polymorphous system existing of different populations of *C.plumosus* can be treated as "adaptive population strategy" determined by the fluctuation of homo-and heterokaryotypes. The phyletic evolution- microevolution differentiation in *C. plumosus* is manifested also by a change in centromeric heterochromatin due to amplification.

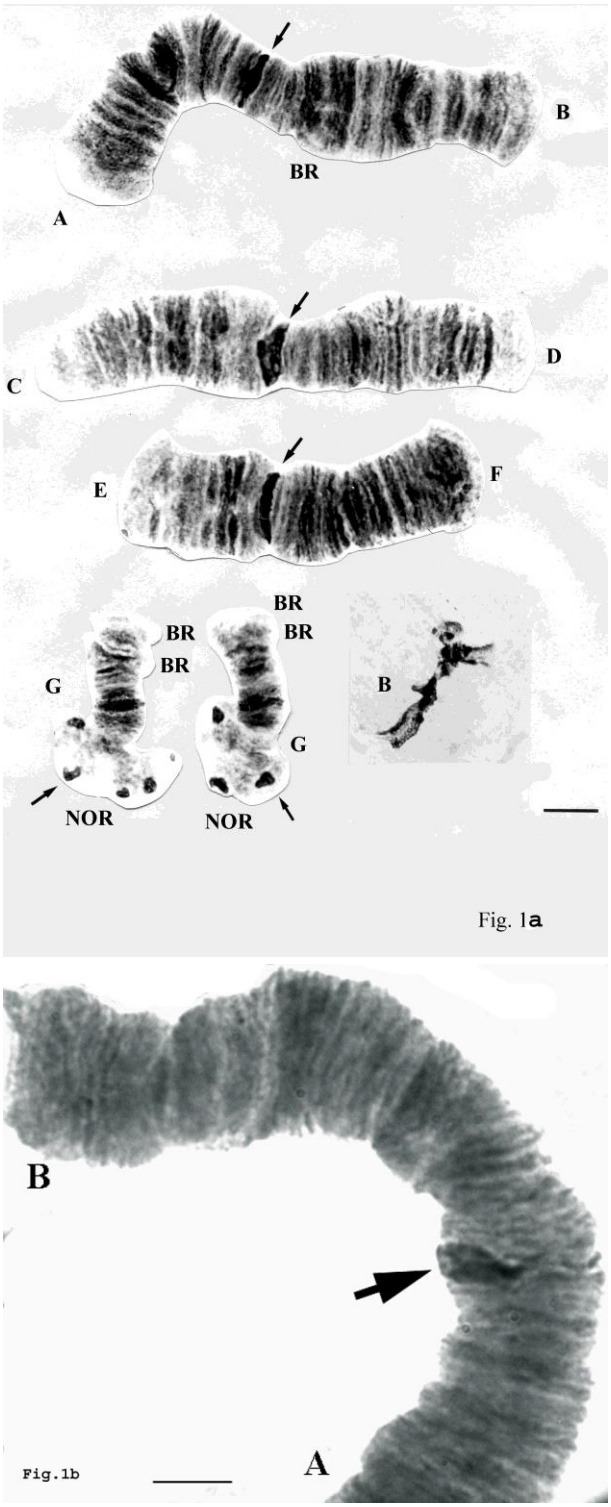

Figure 1. *Polytene chromosomes of Chironomus plumosus L.* ***(a)*** *Chromosomes AB CD EF and G, "B"chromosome;* ***(b)*** *A hybrid chromosome AB (a hybrid between Bulgaria (Plovdiv) and Switzerland (Wohlensee). Bar – 100 µm; NOR – Nucleolar Organizer; BR – Balbiani ring.*

DOI: 10.69085/jbb20151001

RESEARCH ARTICLE

C. plumosus was investigated from various regions of its range (Bulgaria, Hungary, Finland, and Switzerland) (Kiknadze & Siirin, 1991; Michailova, 1994b; Michailova & Krastanov, 2000). Variability was established in the quantity of centromere heterochromatin between investigated populations. The Bulgarian and the Hungarian populations are not distinguished by the amount of centromere heterochromatin. The Swiss and the Finland populations are characterized by large dark centromeric 'C' heterochromatin.

Compared to the other populations these populations have diverged considerably in regard to the amount of heterochromatin due to amplification processes. However, this different centromere heterochromatin has not any isolated effect which was proved by hybridization test (Michailova & Fischer, 1986). When crossing neighbouring or more distant European populations (Bulgaria and Switzerland) the rate of development of embryos, larvae, pupae was almost 100%. The behaviour of the homologous of the hybrid polytene chromosomes does not have the character of an interspecies hybrid. A heterozygous centromere region was discovered in the chromosomes of all hybrids (Figure 1b). Such differences in the appearance of the centromere heterochromatin have been found in various Russian populations as well (Kiknadze & Siirin, 1991).

All these data indicate that *C.plumosus* is at present undergoing intensive karyotype evolution. The high chromosome polymorphism and the changes of the constitutive heterochromatin could be considered as predominant mechanism in the phyletic evolution of the species.

Sympatric speciation

Symbiotopic species

Species which have the same habitat are symbiotopic species, for instance, the sibling species involved in plumosus group: *C. agilis* 1 Shobanov, Djemin, *C. agilis* 2 Kiknadze, Shilova, Kekris, Shobanov, *C. balatonicus* Devai, Wűlker, Scholl, *C. bonus* Shilova, Dzvarsheishvili, *C. borokensis* Kerkis, Kiknadze, Filippova, Gunderina, *C. entis* Shobanov, *C. muratensis* Ryser, Scholl, Wűlker, *C. nudiventris* Ryser, Scholl, Wűlker, *C.usenicus* Loginova, Beljanina. In these species the band pattern of some arms of the polytene chromosomes had been formed by simple or complex fixed homozygous inversions. In both cases these inversions participate in the polymorphous system of the ancestral species - *C. plumosus*. For instance: the chromosome AB of *C. balatonicus* is differentiated from those of *C. plumosus* by complex homozygous inversion (Kiknadze et al., 1991). The homozygous inversion in arm A of *C. balatonicus* has been fixed in that state having passed through several intermediate homozygous inversions, involved in the polymorphous system of *C. plumosus* (Michailova, 1994a). The chromosome CD has gone through transformation, with the homozygous sequence (D4) being part of the polymorphous system of *C. plumosus* and fixed in that state in *C. balatonicus*. In the chromosome EF, a fixed homozygous inversion is observed in the F arm, distinguishing the two species.

Analyses of the polytene chromosomes have revealed also marked differences in the amount and distribution of constitutive heterochromatin in these chromosomes. For instance, chromosome EF shows a great homology in band pattern among species from 'plumosus' group and this chromosome in *C. balatonicus* differs from the standard karyotype of *C. plumosus* by a small distal paracentric inversion only in the arm F (Kiknadze et al., 1991). However, when the 'C' bands of the chromosome of this species are compared with those of *C. plumosus* (a species with a standard karyotype in 'plumosus' group) striking heterochromatin differences can be found (Michailova, 1994b). They are distinguished by the amount and localization of the heterochromatin. In *C. plumosus* (the ancestral species) the heterochromatin is found in centromere regions only. Constant telomere and interstitial 'C' bands were detected in the chromosomes of *C. balatonicus*. There are many bands in *C. plumosus* in euchromatin state which are hetrochromatic in *C. balatonicus*. These results showed that in the derived species, in *C. balatonicus* the heterochromatin is distributed in various sites of the chromosomes: intercalary, in telomere and centromere regions.

The same process as the one described above can be observed in other species of the plumosus group. (Jblonska-Barna & Michailova, 2006; Michailova et al., 2002) formed by homozygotization of their karyotypes. However, in the formation of the *C. nudiventris* (2n = 6) karyotype a translocation of the centromere-telomere fusion type also takes part.

The above examples showed that in the formation of sympatric species of the plumosus group the fluctuating homozygous inversions in *C. plumosus* turn into fixed homozygous - this being considered a characteristic of the derived species.This allows us to consider the polymorphism as a starting point which favours the speciation of

DOI: 10.69085/jbb20151001

RESEARCH ARTICLE

symbiotopic species in family Chironomidae.

There has been no recorded of interspecies hybridisation within this group in the nature. However, laboratory crossing experiments among some sibling species were possible using artificial insemination and these crosses produced variable degree of reproductive isolation. Crosses of *C.plumosus* (female) x *C.balatonicis* (male) produced only 50% fertile of F1 hybrids. Homologous chromosomes of the hybrid *C. plumosus* x *C. balatonicus* are free and independent of each other and they do show a lost attraction to each other. From reciprocal crossing, no egg masses were obtained although spermatozoa were found in the spermateca of the female. It seems that spermatozoa are not able to enter the eggs. These data confirm the occurrence of gamete incompatibility, possible due to a failed insemination reaction. Therefore, between these species there are postmating barriers preventing hybrid survival.

Also, between these species there are effective premationg isolating mechanism. The differences in mating behaviour of sibling species in group plumosus (Ryser et al., 1983) may be an additional mechanism for species differentiation. Furthermore, the larvae of these sibling species live in different niches of water basins (Devai et al., 1983).

Allobiotopic species

The sibling species *Glyptotendipes salinus* Michailova and *G. barbipes* (Staeger) are examples of species which exhibit allobiotopic distribution within a sympatric range. *G.barbipes* larvae can be found in fresh water basins, while those of *G. salinus* are in brackish water. These species have many karyotypic features in common, e.g. the number of chromosomes, the appearance of the centromere region likes a large heterochromatin block, the equal band patterns, and the location of the centromere region (Figure 2) (Michailova, 1987a). Both species most probably originated from a strongly polymorphous species. With the gradual occupation of different habitats - *G. salinus* in brackish water, *G. barbipes* in the freshwater ponds - transformations have occurred in the initial species, resulting in the divergence of both species. The two species are differentiated mainly by homozygous inversion (Michailova, 1987a).

Apart from interspecies differences in chromosomal rearrangements, distinctive chromosome heterochromatin is also a specific cytological character. There are two primary processes occurring in the formation of these species: (1) transformation of euchromatin; and (2) amplification of heterochromatin. The process of transformation is observed in sibling species *G. barbipes* and *G. salinus* (Michailova, 1987b) - many bands which are in a euchromatic state in *G.salinus* become in heterochromatic state in *G.barbipes*. It is possible that these staining differences reflect either some heterochromatin protein modification between species or differential amount of repetitive DNAs, or both.

The significance of heterochromatin amplification is clearly seen in species of *Gliptotendipes*. Significant differences were found in the amount of centromere heterochromatin - significant higher in *G.barbipes* (Michailova & Nikolov, 1992). However, these species were distinguished not only by the amount of centromeric heterochromatin but also by the quality of the heterochromatin. C-heterochromatin of *G. barbipes* consists of two different C-bands; (1) dark C-bands at the periphery of the centromere of the chromosomes which correspond to satellite II DNA; and (2) pale C-bands in the moddle of the centromere, corresponding to the satellite I DNA. Differentiation in the centromere region of the chromosomes of *G. salinus* is weakly expressed (Michailova, 1987b). It is quite possible that these insects share a common library of satellite sequences which are amplified to various degrees so that in each species one may see different amounts of heterochromatin.

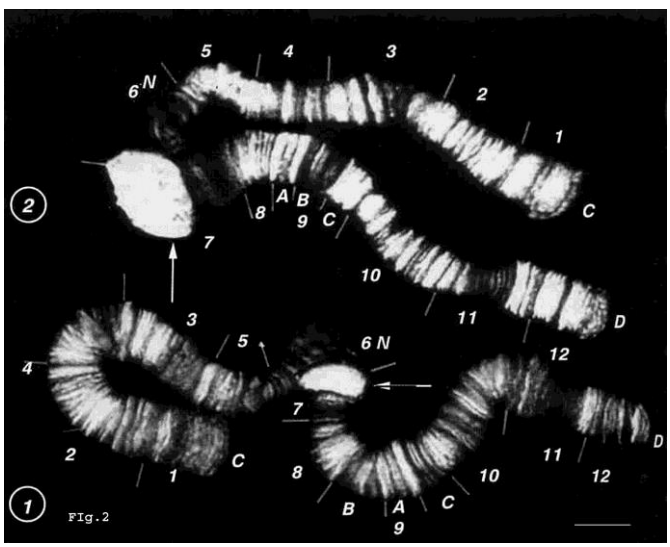

Figure 2. *Polytene chromosomes of genus Glyptotendipes Mg. (1) Glyptotendipes salinus Michailova; (2) Glyptotendipes barbipes (Staeger). Bar - 100 µm.*

DOI: 10.69085/jbb20151001

RESEARCH ARTICLE

During species divergence (*G. barbipes* is phylogenetically the younger of the two species) mutations could be accumulated within the members of cluster repeats of this heterochromatin and could account for the observed heterogeneity of cytological appearance in the *G. barbipes* heterochromatin. Therefore, the heterochromatin can be considered a dynamic element during speciation.

There has been no recorded of interspecies hybridization between these species in nature. However, laboratory crossing experiments among these sibling species were possible using artificial insemination and produced variable degrees of reproductive isolation. Hybridization was successful only in one direction (*G. salinus* female X *G. barbipes* male). Egg hatchability of this cross was about 70%. Larval viability was reduced so that about 27% of the eggs developed into adults. Although the banding patterns were very similar the giant chromosomes of the hybrids showed almost complete asynapsis, a phenomenon which must be caused by genic changes which may not be reflected in the banding patterns. Therefore, between these species there are postmating barriers preventing hybrid survival. Also, between these species there are effective premating isolating mechanisms. The both species coexist sympatrically in certain areas without merging: *G. salinus* occurs in brackish water, while G. barbipes prefers fresh water.

Homosequental species

Chironomus riparius Mg. and *Chironomus piger* Strenzke are homosequental species as they have the same chromosome set (2n = 8) and banding patterns in all chromosomes. The both species distinguished by their phylogeny: phylogenetically younger is *C. riparius*. They differences by the families of tandem-repetitive and minisatellite sequences for instance, Cla elements, Hinf and Alu elements. *C.riparius* has significantly more repetitive DNA sequence elements than *C.piger* (Schmidt, 1984; Hankeln et al., 1994). For instance, in *C.piger* genome was established one signal of Alu element, while in *C.riparius* genome there were 22 (Figure 3a, b).

Also, both species are differentiated on the amount and localization of the heterochromatin (Michailova et al., 2009; Ilkova et al., 2013). The studied done by these authors showed that the insertions sites of some transposable elements detected by FISH is approximately two times higher in *C.riparius* than in *C.piger*.

How does the process of sympatric formation of the species occur? Initially, groups of individuals, each of them with a definite genotypic structure, developed in the population. Depending on the ecological conditions, the standard homokaryotype, heterokaryotype or inverted homokaryotype is predominant.

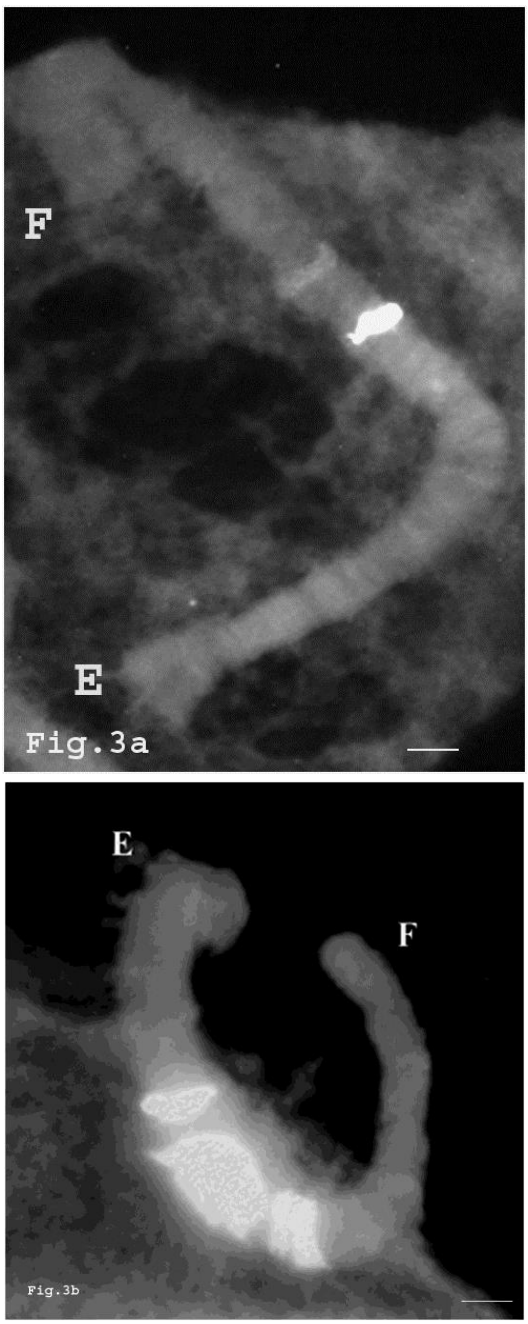

Figure 3. *Localization of repetitive DNA element (Alu).* ***(a)*** *Chironomus piger Strenzke;* ***(b)*** *Chironomus riparius Mg. Bar - 100 μm.*

DOI: 10.69085/jbb20151001

RESEARCH ARTICLE

Thus chromosome changes appear to be a protective mechanism for the gene pool of the population. For instance, in heterozygous with adaptive value, crossing-over is suppressed, thus allowing the preservation this co - adapted gene complexes in the population. Later on, as a result of positive assortative mating, one or other of the groups with a particular genetic structure is stabilized. In this way, the heterokaryotypes after many generations pass into homokaryotypes. Under certain conditions, new to the species homozygous inversions with adaptive priority are selected, and spread in the population and become fixed in it. The occurrence of a new homozygous karyotype is associated with the restriction of panmixia with the initial alternative homozygous form. This brings about the enhancement of reproductive barriers between alternative homozygous groups in a population and to their independent evolution, notwithstanding the fact that they live in the same range. Thus, at a certain time polymorphism turns into monomorphism; for instance, in the plumosus group some homozygous inversions taking part in the polymorphous system of *C. plumosus* become constant characteristics of the karyotypes of sibling species.

Allopatric speciation

This type of speciation can be observed in species geographically isolated. Good expamles are *C.plumosus* (Palaearctic populations) and *C. vancouveri* (Canada population). The both species are distinguished by simple fixed homozygous inversion in chromosome EF as well as by the amount and localization of the heterochromatin (Michailova, 1994b).

The comparative analysis of polytene chromosomes and reproductive relations of Palaearctic species – *C.plumosus* with the Nearctic species *C. vancouver* showed that the generator of speciation in this group appears to be the Palaearctic species *C. plumosus* with a standard karyotype (Michailova & Fisher, 1984). Results from cytotaxonomic and hybridization tests indicate closer similarity between the allopatric species than between the sympatric ones. In crossing *C. plumosus* (Bulgaria) and *C. vancouveri* (Canada) fertile hybrids were produced in both directions of crossing but with a low fertility percentage- less than 50%. Despite of the fact that the homologues in the polytene chromosomes of hybrids are in a state of asynapsis, they are closely oriented to each other (Figure 4). In some sections of the polytene chromosomes great similarities in banding patterns were observed without synapsis of the homologues, a phenomenon, indicted that some genic changes were accompanied the divergence of the species, which are not reflected in the morphology of the polytene chromosomes. The gene flow between the both species was restricted and they are diverged independent from each other. Such type of speciation has been observed in *C.tentans* from Nearctic population which allows for the new species to be described in this population (Shobanov et al., 1999).

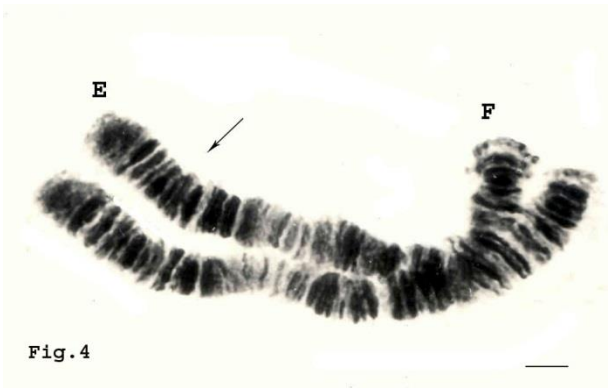

Figure 4. *A hybrid chromosome EF of C.plumosus L. x C.vancouveri Michailova, Fisher. Bar - 100 µm.*

Natural introgressive hybridization

Introgressive hybridization is one of the important paths of speciation in Chironomidae family, especially genus *Glyptotedndipes* (Michailova 1998; Michailova & Petrova, 1984; Michailova & Contreras-Lichtenberg, 1995). It is produced by crossing of hybrid obtained between two species (F1) with one of the initial parent form. In this process is going the incorporation of genes of one species into gene pool of another species by hybridization and backcrossing.

On the Black Sea coast, near the region of Burgass, a population of hybrid origin was found. This was a hybrid between closely related species *G.pallens* Mg. and *G.glaucus* Mg.. Some specimens from this locality carried a hybrid chromosome G.: one homologous from *G.glaucus* and other from *G.pallens.* The origin of this hybrid can be explained by introgression: F1x *G.glaucus* (Figure 5).

In other populations (Bulgaria, Shabla lake) and Hungary (Velencea) also a hybrid produced by introgression was observed (F1 x G.*pallens*). The introgression was realized as backcrossing of F1 with one of the parents. Somewhere in the past it produced hybrids between two species: *G.pallens* and *G.glaucus*. From these, different types of gametes could be formed. A hybridization between gametes designated with "X1" and that of *G.glaucus* had happened and individuals

DOI: 10.69085/jbb20151001

RESEARCH ARTICLE

with a hybrid chromosome G, (one homologue as *G.glaucus* and other homologue as *G.pallens*) could be seen. Chromosomes AB, CD and EF corresponded to those of *G.glaucus.*

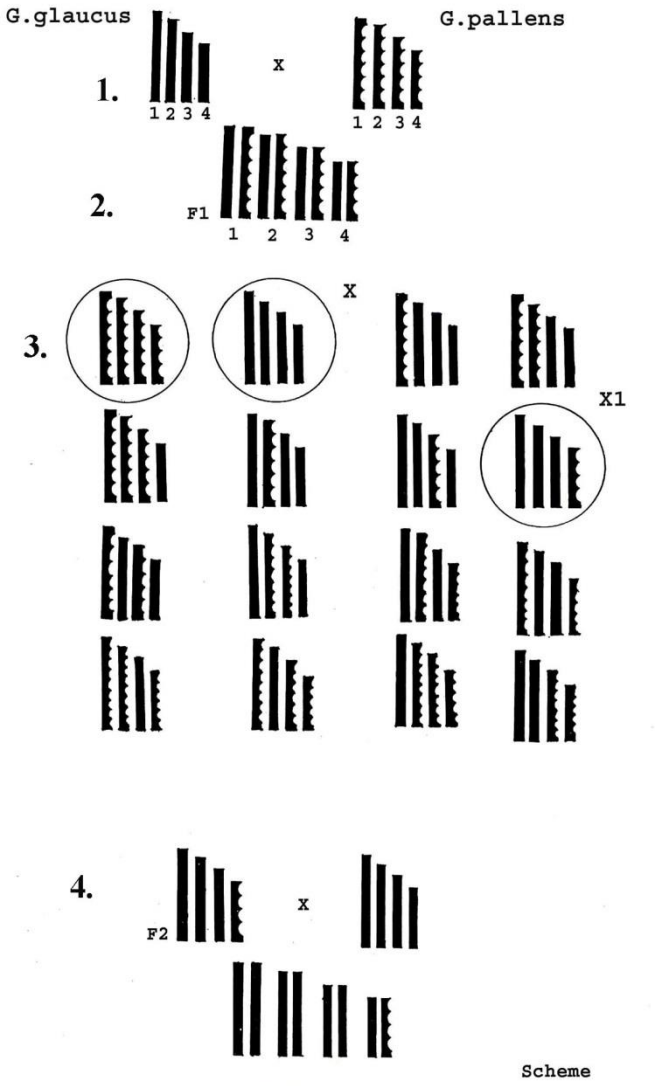

Figure 5. *A process of introgression between two closely related Glyptotendipes species: G. pallens Mg. and G. glaucus Mg. 1. Polytene chromosomes of G.glaucus and G.pallens. 2. A hybrid between G.glaucus and G.pallens. 3. Different gametes produced by a hybrid of G.glaucus and G.pallens 4. Introgression - gametes indicated by "X1" combined with gametes of G.glaucus and produced a hybrid with a hybrid chromosome G.*

The zones with hybrid populations are always on localities, where contacts exist between two species. The stability of a hybrid population can be explained by hybrids superiority. In this zone the hybrids have superior fitness and the hybrid stability can be related to the amount of genetic distance between the hybridization taxa (Graham, 1992). In fact the hybridized species are closely related species, recently derived from a common ancestor and still sharing much of their genomic organization. Such path of speciation was observed in Baikal Lake in other species of genus *Glyptotendipes* (Michailova & Petrova, 1984).

Conclusion

Three types of speciation can establish in Chironomidae family: sympatric, allopatric and introgression:

- Many and more complicated chromosome rearrangements (overlapping homozygous inversions, fusions, etc.) are involved in **sympatric speciation**, i.e., the species with overlapping ranges are characterized by more complex chromosome changes.

- In species with **allopatric speciation** rearrangements in the karyotype are also observed but these are not so complicated; the reproductive isolation is also due to the distance between the populations.

- In the **introgressive speciation** each species is characterized by a unique integrated internal system of genetic balance, which is not destroyed by recombination. These species can coexist together even in the presence of considerable interbreeding. Each species remains open to the acquisition of small amounts of genetic variability from the other. The evidence indicates that the species have subsequently maintained reproductive isolation but have retained some "complex chromosomes" consisted of chromosomes of both species. In same cases, however can be produced a species with a hybrid origin.

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RESEARCH ARTICLE

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