Metaphase I orientation of Robertsonian trivalents in the water-hyacinth grasshopper, *Cornops aquaticum* (Acrididae, Orthoptera)

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**Abstract**

Trivalents resulting from polymorphic Robertsonian rearrangements must have a regular orientation in metaphase I if the polymorphisms are to be maintained. It has been argued that redistribution of proximal and interstitial chiasmata to more distal positions is necessary for a convergent orientation, the only one that produces viable gametes. *Cornops aquaticum* is a South-American grasshopper that lives and feeds on water-hyacinths, and has three polymorphic Robertsonian rearrangements in its southernmost distribution area in Central Argentina and Uruguay. The orientation of trivalents in metaphase I, the formation of abnormal spermatids and the frequency and position of chiasmata in the trivalents, was analysed in a polymorphic population of *C. aquaticus*. In this study we observed a correlation between the number of trivalents with the frequency of abnormal spermatids; additionally, the number of chiasmata, especially proximal and interstitial ones, was strongly correlated with the frequency of the linear orientation. Therefore we confirmed our previous assumption, based on other evidence, that the chiasmata redistribution in fusion carriers is essential to the maintenance of the polymorphisms.

**Key words: Cornops aquaticum, Robertsonian rearrangements, trivalents, metaphase I orientation.**

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cline were found in the southernmost extreme of *C. aquaticum* geographic distribution (Mesa, 1956; Mesa et al., 1982; Colombo, 2008). The chromosomes involved in these centric fusions have their chiasma distribution severely affected, shifting from proximal and interstitial to more distal positions (Colombo, 2007). In this work, we analysed the relationship between proximal and interstitial chiasma frequency and linear orientation. We also studied the correlation between non-convergent orientation and the formation of abnormal spermatids.

Twenty-one out of the 27 males used in this study were from two highly polymorphic populations of *Cornops aquaticum* from Zárate and Baradero, Argentina, on the Paraná River, during the Austral Summers of 2005, 2006 and 2007. Other three males from a mostly monomorphic population of *C. aquaticum* (Tigre, close to Buenos Aires) were used and represented the “zero trivalent” class. The males were dissected and their testes were fixed in 3:1 ethanol:acetic acid. Cytological preparations were made by squashing some follicles of the testis in propionic haematoxylin. Metaphase I plates were scored for trivalent chiasma frequency and position and for trivalent orientation (linear or convergent). In order to classify chiasma positioning, the arms of the trivalents were divided in three. Chiasma located in the proximal third of the chromosome arm were called “proximal” (P), those located in the intermediate third were classified as “interstitial” (I) and those present in the distal third were called “distal” (D). Signs of structural tension were taken into account when analysing trivalent orientation. If no tension was observed, the cell was considered to be in prometaphase I and was not computed. This consideration is important because the inclusion of these cells in the analysis would lead to an overestimation of linear orientation. In order to assess abnormal spermatids, we counted the number of “centriolar adjuncts” (the root of the future flagellum) establish whether a spermatid was haploid, diploid or polyploid. Standard orientation and the number and position of chiasmata (Table 1). Normality of the variable “percentage of linear orientation” was assessed by means of a Kolmogorov-Smirnov test which was not significant (p = 0.09788), thus not ruling out normality. Total chiasma frequency among individuals with one, two and three trivalents and without heterozygous fusions were compared with respect to the formation of diploid or tetraploid spermatids (Table 2). The variable “proportion of abnormal spermatids” did not show a normal distribution. The Kolmogorov-Smirnov test rendered non-significant values (p = 0.1024319) when the variable was transformed to log10, thus showing normal distribution. The regression of “percentage of abnormal spermatids” on “number of trivalents” was significant (p = 0.019919). The regression of “percentage of abnormal spermatids” on “number of trivalents” was significant (p = 0.019919). The regression of “percentage of abnormal spermatids” on “number of linear orientation” was not significant (p = 0.591304).

*Cornops aquaticum* is not a favourable material for the study of aneuploidy in metaphase II, since cells in these meiotic stage are scarce and the plates are of bad quality.
Trivalent orientation in *Cornops aquaticum*

**Table 1 - Metaphase I orientation of trivalents and chiasma frequency in *Cornops aquaticum***

| N     | L     | P | I | D | P' | I' | D' |
|-------|-------|---|---|---|----|----|----|
| B06002| 34    | 5.88 | 0 | 1.50 | 1.00 | 0.06 | 0.31 | 1.66 |
| B06003| 186   | 0.54 | 0 | 2.00 | 0.04 | 0.04 | 1.93 |
| B06001| 91    | 5.49 | 1.20 | 0.20 | 1.80 | 0.13 | 0.21 | 1.81 |
| Z06001| 140   | 7.14 | 0.30 | 0.20 | 1.80 | 0.04 | 0.07 | 1.93 |
| Z06002| 138   | 2.90 | 0.50 | 1.00 | 1.25 | 0.04 | 0.44 | 1.60 |
| Z06005| 123   | 2.44 | 0 | 0.67 | 2.00 | 0.03 | 0.14 | 1.96 |
| Z06004| 175   | 0.57 | 0 | 2.00 | 1.00 | 0.37 | 0.59 | 1.81 |
| Z07001| 274   | 2.92 | 0.87 | 0.75 | 1.25 | 0.07 | 0.48 | 1.63 |
| Z07002| 178   | 2.25 | 0.25 | 0.50 | 1.50 | 0.06 | 0.04 | 1.87 |
| Z07007| 93    | 5.38 | 0.80 | 0.10 | 1.20 | 0.19 | 0.41 | 1.83 |
| Z07004| 106   | 3.77 | 0 | 0.25 | 1.75 | 0.01 | 0.03 | 2.00 |
| Z07005| 148   | 0.68 | 0.27 | 0.88 | 1.75 | 0.24 | 0.84 | 1.57 |
| Z07008| 236   | 4.66 | 0.63 | 0.82 | 1.36 | 0.12 | 0.49 | 1.42 |
| Z06013| 74    | 8.11 | 0.33 | 1.50 | 1.50 | 0.06 | 0.91 | 1.69 |
| Z05027| 204   | 4.90 | 0.60 | 0.20 | 1.40 | 0.11 | 0.20 | 1.80 |
| Z05024| 379   | 2.64 | 0 | 0.60 | 1.80 | 0.06 | 0.11 | 1.94 |
| Z05005| 284   | 13.73 | 0.13 | 0.67 | 1.33 | 0.08 | 0.35 | 1.79 |
| Z05020| 101   | 4.95 | 0.63 | 0.40 | 1.60 | 0.29 | 0.78 | 1.46 |
| Z05004| 254   | 3.15 | 0.63 | 0.22 | 1.13 | 0.19 | 0.56 | 1.40 |
| Z05012| 58    | 8.62 | 1.20 | 1.20 | 1.60 | 0.08 | 0.70 | 1.35 |
| Z05010| 83    | 7.23 | 0.33 | 0.50 | 2.00 | 0.03 | 0.19 | 1.78 |

N = Total number of cells; L = percentage of linear orientation; P, I, D = proximal, interstitial and distal frequency of chiasma in trivalents with linear orientation; P’, I’, D’ = proximal, interstitial and distal frequency of chiasma in trivalents with convergent orientation.

(Figures 1C and D). Only five out of 149 metaphase II plates analysed presented aneuploidy. Anaphase I cells were also rare but their quality was much better. A total of 53 anaphase I cells were analysed and the segregation of trivalents was always normal, with the submetacentric moving towards one pole and the two corresponding acrocentrics migrating towards the other.

Centric fusions are usually accompanied by extensive chiasma repatterning and shifting of chiasma position in acrocentric bivalents to more distal positions in Robertsonian bivalents or trivalents (Bidau, 1990; Colombo, 1989, 1990, 1993, 2007; Davison and Akeson, 1993; Dumas and Britton-Davidian, 2002; Hewitt and Schroeter, 1968). However, the cause for chiasma redistribution in Robertsonian bivalents may not be the same as in Robertsonian trivalents. When chromosome races are compared, the race with the lowest diploid number frequently has less (proximal) chiasma than the race with a higher diploid number (Davison and Akeson, 1993; Dumas and Britton-Davidian, 2002). We attribute this difference to interference across the centromere. In fact, ever since Mathers work (1938), the centromere has been considered a barrier to the operation of interference. Nevertheless, Colombo and Jones (1997) demonstrated that interference does operate across the centromere in the grasshopper species *Leptysma argentina* and *Chorthippus brunneus*. The same was demonstrated in humans with an improved statistical method (Broman and Weber, 2000).

Another factor must operate in trivalents. When there is a trivalent, the synaptonemal complex (SC) is interrupted at the level of the centromere. It has been frequently suggested that either the operation of interference needs a complete SC (Sym and Roeder, 1994) or that SC formation needs the action of interference. Anyway, there is consensus that both SC formation and interference are related. It thus follows that interference should not operate across the centromere in trivalents.

It must be pointed out that in trivalents, it is always **polymorphic**, and never polytypic or spontaneous centric...

**Table 2 - Metaphase I orientation of trivalents in *Cornops aquaticum* and formation of abnormal spermatids.**

| N     | L     | S  | A   | N  |
|-------|-------|----|-----|----|
| B06002| 34    | 5.88 | nd | nd | 1  |
| B06003| 186   | 0.54 | 1583| 1.08| 2  |
| B06001| 91    | 5.49 | 986 | 2.60| 3  |
| Z06001| 140   | 7.14 | 1962| 1.08| 1  |
| Z06002| 138   | 2.90 | 1059| 2.32| 1  |
| Z06005| 123   | 2.44 | 787 | 6.35| 1  |
| Z06004| 175   | 0.57 | 1389| 1.61| 1  |
| Z07001| 274   | 2.92 | 1136| 1.97| 3  |
| Z07002| 178   | 2.25 | 2191| 0.23| 2  |
| Z07007| 93    | 5.38 | 1196| 1.10| 1  |
| Z07004| 106   | 3.77 | 756 | 3.00| 2  |
| Z07005| 148   | 0.68 | 1093| 2.05| 2  |
| Z07008| 236   | 4.66 | 1310| 1.71| 2  |
| Z06013| 74    | 8.11 | nd | nd | 2  |
| Z05027| 204   | 4.90 | nd | nd | 2  |
| Z05024| 379   | 2.64 | nd | nd | 2  |
| Z05005| 284   | 13.73| nd | nd | 2  |
| Z05020| 101   | 4.95 | 476 | 5.78| 3  |
| Z05004| 254   | 3.15 | 1387| 2.36| 3  |
| Z05012| 58    | 8.62 | 2116| 2.17| 1  |
| Z05010| 83    | 7.23 | 1896| 0.90| 1  |
| Z06003| -     | -   | 967 | 1.63| 0  |
| Z06007| -     | -   | 962 | 0.93| 0  |
| Z06006| -     | -   | 1623| 0.67| 0  |
| T06017| -     | -   | 1198| 0.92| 0  |
| T06015| -     | -   | 1553| 0.39| 0  |
| T06016| -     | -   | 1235| 0.64| 0  |

N = Total number of cells; L = percentage of linear orientation; S = total number of recorded spermatids; A = percentage of abnormal spermatids; N = number of trivalents.

Total 3359 21984
fusions that present chiasma redistribution to more distal positions. Indeed, chiasma frequency and position are usually unchanged in spontaneous centric fusions (Colombo, 1987; López-Fernández et al., 1984; Sannomiya, 1968; Southern, 1967). Concurrently, when studied, more than 36% of metaphase I plates showed linear orientation. (Colombo, 1987; Kayano and Nakamura, 1960; López-Fernández et al., 1984; Teoh and Yong, 1983). Bidau et al. (2001) studied chiasma frequency and distribution in a hybrid zone of Mus musculus. In this case, the individuals studied were natural hybrids between a race with four fixed Robertsonian rearrangements and another one with all acrocentric chromosomes. The race with four submetacentric bivalents showed a decreased number of proximal and interstitial chiasmata, as expected, but hybrids showed trivalents with increased proximal and interstitial chiasma frequencies. The orientation of trivalents in metaphase I was not studied.

We thus conclude that chiasma repatterning towards more distal positions in the case of Robertsonian trivalents is restricted to polymorphic conditions. The suppression of proximal and interstitial chiasmata is clearly adaptive for the maintenance of the polymorphism, given that proximal and interstitial chiasmata are correlated with the frequency of linear orientation and thus with the origin of aneuploid and/or polyploid gametes (Bidau and Mirol, 1988, Mirol and Bidau, 1991, 1992). In this study, the correlation between the proportion of abnormal spermatids and the percentage of linear orientation was not significant. However, we were unable to correlate the formation of aneuploid spermatids and the linear orientation because we only identified polyploid spermatids. Nevertheless, we can assume that linear orientation leads to the formation of aneuploid sperm, even if in some cases reorientation may occur.

Whether or not the reorganisation of chiasma frequency and position was triggered by natural selection favouring the maintenance of the polymorphism or by the rearrangement itself is largely a matter of speculation. If the latter is true, only preadapted centric fusions (i.e., those that cause chiasma repatterning) would survive in a polymorphic state. In either case, the fact that no abnormal anaphase I and very few aneuploid metaphases II had been detected reflects the stability of the polymorphisms herein studied.

Lagging chromosomes or trivalents may result in polyploid spermatids (John et al., 1983). We thus studied polyploid spermatids in order to evaluate the degree of infertility caused by cytological heterozygosis. This approach can only detect the infertility caused by polyploid spermatids and that due to aneuploid spermatids should be studied with more sophisticated techniques (Lowe et al., 1996). The analysis revealed a correlation between the percentage of polyploid spermatids and the number of trivalents (Figure 2), thus confirming the relationship between these two variables. This result coincides with those found by Bidau and Mirol (1988) in Dichroplus pratensis. The percentage of polyploid spermatids was extremely low, corroborating the stability of the polymorphisms herein studied.

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