CLADISTIC ANALYSES OF MOSQUITO CHROMOSOME DATA IN *ANOPHELES* SUBGENUS *CELLIA* (DIPTERA: CULICIDAE)

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ABSTRACT. Cladistic methodology is discussed in the context of mosquito chromosome inversions, and the available data within *Anopheles* subgenus *Cellia* is reinterpreted. Within the Myzomyia Series, a sister group relationship is corroborated between the Wellcomei Group and the clade Funestus Group + Rivulorum Group, leaving the Demeilloni Group as a clade of uncertain position. Within the Neocellia Series, only two groups are recognized as monophyletic: the Maculatus Complex and a clade consisting of *An. rufipes* + *An. maculipalpis* + *An. pretoriensis*. For the Gambiae Complex of the Pyretophorus Series, the sister group relationships *An. bwambae* + *An. melas* and *An. gambiae* + *An. merus* are confirmed, although outgroup data is almost absent, making hypotheses highly premature.

INTRODUCTION

About a decade ago, White (1980) mentioned that there is much to exploit in mosquito population genetics as interpreted from karyotype rearrangements, and he suggested that "the aim may be to plot phylogenetic relationships." With regard to chromosome inversion data, this has been done for selected members within three of the six series presently recognized in *Anopheles* Meigen subgenus *Cellia* Theobald: the Gambiae Complex of the Pyretophorus Series (Coluzzi and Sabatini 1967, 1968, 1969; Coluzzi et al. 1979; G.B. White 1973, 1985), the Funestus Group and relatives in the Myzomyia Series (Green 1982) and the majority of species in the Neocellia Series (Green and Baimai 1984, Green et al. 1985). These groups have been favored as they contain some of the most potent disease vectors, especially of malaria but also of lymphatic filariasis.

White (1978) postulated a "tentative chromosomal phylogeny" of the Maculipennis Complex and some related taxa of the subgenus *Anopheles*, but this was "merely a tentative attempt to construct a framework which might support more definitive research on the phylogenetics of *Anopheles sensu stricto*." However, the paper does not contain sufficient data to be analyzed in the present context.

Even within *Cellia*, only sparse data are available, and this has been considered an obstruction to the formulation of phylogenetic hypotheses. Seetharam and Chowdaia (1974) stated that because few species had been examined in subgenus *Cellia*, "any attempt to explain the evolutionary relationships based on the chromosomal comparison alone ... would be arbitrary," and Green (1982) was of the opinion that "it seems unnecessary to take a firm stand on these scant data since there are many more members of ... Myzomyia as yet unknown cytologically." However, although we always should consider bringing in more data, we still need to believe in whatever data we have and be careful and explicit in the way we derive our hypotheses. With the important contribution of Green et al. (1985), a combined analysis of both the Myzomyia and the Neocellia Series is made possible, and the present paper is a discussion of cladistic methodology and a reinterpretation of data available on chromosome inversions within *Anopheles* subgenus *Cellia*.

METHODS

Character matrices of the present paper were constructed by compilation of the data from Coluzzi et al. (1979), G.B. White (1973, 1985), Green (1982), Green and Baimai (1984), Green et al. (1985), Petrarca et al.

(1987) and Subbarao et al. (1988). All phylogenetically uninformative inversions were omitted, i.e., those for which all or all but one of the scorings were identical. States were characterized as presence or absence without reference to probable apomorphy and plesiomorphy, i.e., without reference to the designations of "standard" or "inverted." This must be deduced from the cladograms and character distributions given in Figs. 1,2 and Tables 2,4. Uninterpreted homologies equal "character state unknown" and have been scored as "-." Formal names of characters are taken from the original sources, although chromosome arm designations have been modified according to Green and Hunt (1980). Matrices were analyzed on an IBM PS/2-30 with the computer package Hennig86 (version 1.5, copyright J.S. Farris 1988), which is an interactive program for phylogenetic analysis. Cladograms were generated with the "mh*;bb*;" option. This will construct several initial cladograms by adding terminal taxa in several different sequences and apply extended branch-swapping to each. Only the shortest cladograms are retained. This was followed by successive weighting: "xs w;mh*;bb*;xs w;mh*;bb*;" etc., until weights no longer changed. This procedure will set the character's weight according to its fit to the cladogram in question, scaled to lie in the range 0–10. That is, characters showing less homoplasy will be given higher weight. For further information, see the documentation by Farris (1988).

The formal taxonomy has been updated from the data sources used by replacing "*An. aruni*?" of Green (1982) with *An. vaneedeni* Gillies and Coetzee as made available by Gillies and Coetzee (1987), and incorporating the names proposed by Rattanarithikul and Green (1987) and Rattanarithikul and Harbach (1991) within the Maculatus Complex. *Anopheles culicifacies* Giles is a complex of four species (Subbarao et al. 1983, 1988; Milligan et al. 1986) and was scored as *An. culicifacies* species a, b, c and d. The Subpictus Complex was scored as a single terminal taxon for the present use as outgroup.

Green et al. (1985) mention "a small terminal inversion (as yet not designated) ... found in all other series [than Myzomyia] within Cellia." This inversion (**2*** in Table 2) identifies the Myzomyia Series as the ingroup and the Neocellia Series as the outgroup.

The Subpictus Complex is the outgroup for the Gambiae Complex as it is the only taxon known to possess homologous segments for which at least two character states exist in the ingroup. Narang et al. (1973) mapped the larval salivary polytene chromosomes of *An. subpictus* Grassi and found the segments $+^{a}$, $+^{b}$ and $+^{bc}$ of chromosome 2 (given as 2R), which are present fixed or floating in all species of the Gambiae Complex. Hence, *An. subpictus* may be scored for the two latter inversions (actually for the absence of the inverted condition), presence of $+^{a}$ being phylogenetically uninformative as only *An. arabiensis* Patton carries the inverted condition.

Methodological research is required on the principles for using chromosome inversion data in phylogenetic analyses. Green (1982) and Green et al. (1985) point out that the problem is somewhat different from traditional data treatment in that ancestors may be polymorphic for a given character. Therefore, apparent parallelisms may be expected to be more frequent, which is important for groundplan estimates of hypothetical ancestors.

RESULTS

Characters and character matrices are given in Tables 1–4. Running the Myzomyia-Neocellia matrix resulted in a total of 222 equally most parsimonious cladograms, which for convenience were reduced to the Nelson consensus cladogram shown in Fig. 1 (i.e., a condensed cladogram containing only those clades which are common to all the original cladograms). The matrix for the Gambiae Complex gave a total of three cladograms (Fig. 2), the Nelson consensus cladogram of which is identical to that in Fig. 2C. Character states for non-terminal clades are shown in Tables 2 (Myzomyia + Neocellia Series) and 4 (Gambiae Complex).

DISCUSSION

Chromosome rearrangements *a priori* are considered phylogenetically valuable because

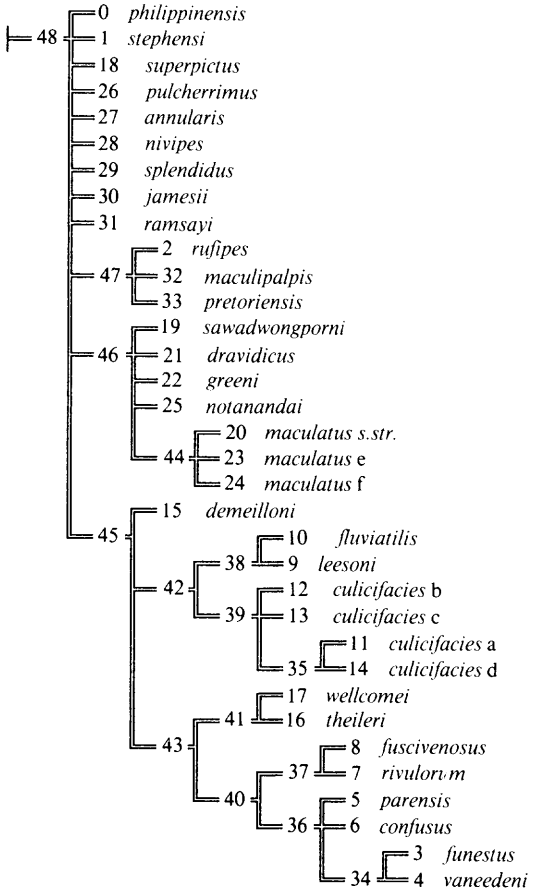

Fig. 1. Myzomyia + Neocellia Series. Nelson consensus cladogram; clade numbers refer to Tables 1 and 2.

these provide one of several explanations of speciation events (M.J.D. White 1973) and thus have a potential impact on macroevolution. Farris (1978) argued that chromosome inversion data fit into a phylogenetic analysis, i.e., inversions are valid characters. Characters form the basis of any phylogenetic analysis because they are transformation series. Any somatic chromosome of a diploid cell has a homolog, which means that inversion characters in diploid organisms basically exist in three states: homozygous for the "standard" condition, homozygous for the "inverted" condition, and heterozygous. This may be further elaborated if inversion frequencies are considered phylogenetically informative (see discussion by Swofford and Berlocher (1987) and references therein). Only one transformation series is possible, namely from the standard homozygote to the heterozygote to the inversion homozygote (or vice versa). Note that this three-state configuration with a linear transformation series is identical to a purely binomial scoring for presence/absence of the "standard" and "inverted" conditions. It should be stressed that "standard" and "inverted" in this context often are arbitrary terms, although it may be desirable on conceptual grounds to denominate the plesiomorphic condition as standard. The hypothetical nature of apo- versus plesiomorphy, however, means that (hypothetical) directions of transformation series are liable to change, and more neutral terms become desirable. An inverted segment will obtain the same orientation, e.g., relative to the centromere, as its

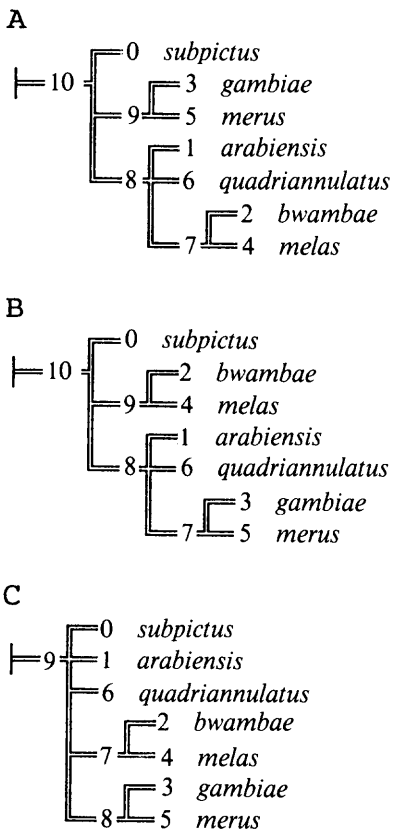

Fig. 2. Gambiae Complex. Equally most parsimonious cladograms. Note that C is identical to the Nelson consensus cladogram of all three cladograms. Clade numbers refer to Tables 3 and 4.

Table 1. Myzomyia + Neocellia Series, character matrix. Scorings are taken from the original sources indicated in Table 2.

Anopheles	Characters
0 *philippinensis*	-- 2------2---------- -------- ----- - 00200000 2022 00 000
1 *stephensi*	-- 2------2---------- -------- 00000 - 20000000 0000 00 100
2 *rufipes*	-- 200--0-2--0-----00-0 00-0000- 00000 - 02000000 2200 20 002
3 *funestus*	-- 02202220000000000000 22220000 20000 - -------- ---- -- ---
4 *vaneedeni*	-- 02202220000000000000 22220000 20000 - -------- ---- -- ---
5 *parensis*	-- 02202220000000000000 02220000 20000 - -------- ---- -- ---
6 *confusus*	-- 02202220000000000000 02220000 20000 - -------- ---- -- ---
7 *rivulorum*	-- 00000000220000000000 00220000 02000 - -------- ---- -- ---
8 *fuscivenosus*	-- 00000000220000000000 00220000 02000 - -------- ---- -- ---
9 *leesoni*	-- 00000002002200200000 00002002 00000 - -------- ---- -- ---
10 *fluviatilis*	-- 00000002002200200000 00002002 00000 - -------- ---- -- ---
11 *culicifacies* a	22 00010002002222000000 00002000 00000 - 0------- 0--- 00 ---
12 *culicifacies* b	00 00000002002222000000 00002000 00000 - 0------- 0--- 00 ---
13 *culicifacies* c	00 00000002002222000000 00002000 00000 - 0------- 0--- 00 ---
14 *culicifacies* d	22 00020002002222000000 00002000 00000 - 0------- 0--- 00 ---
15 *demeilloni*	-- 00000002200000000000 00000000 00000 - -------- ---- -- ---
16 *theileri*	-- 00000000000200022222 00000220 00222 - -------- ---- -- ---
17 *wellcomei*	-- 00000000000000202222 20000221 00222 - -------- ---- -- ---
18 *superpictus*	-- 2------2---------- ------- ----- - 20000000 2000 00 000
19 *sawadwongporni*	-- 2------2---------- ------- ----- 2 20000000 2000 02 220
20 *maculatus s.str.*	-- 2------2---------- ------- ----- 1 20022222 2000 02 220
21 *dravidicus*	-- 2------2---------- ------- ----- 0 20000000 2000 02 220
22 *greeni*	-- 2------2---------- ------- ----- 2 20000000 2000 02 220
23 *maculatus* e	-- 2------2---------- ------- ----- 1 20022222 2000 02 220
24 *maculatus* f	-- 2------2---------- ------- ----- 0 20022222 2000 02 220
25 *notanandai*	-- 2------2---------- ------- ----- 2 20000000 2000 02 220
26 *pulcherrimus*	-- 2------2---------- ------- ----- - 00200000 2022 00 000
27 *annularis*	-- 2------2---------- ------- ----- - 00200000 2022 00 000
28 *nivipes*	-- 2------2---------- ------- ----- - 00200000 2022 00 000
29 *splendidus*	-- 2------2---------- ------- ----- - 00000000 2000 00 000
30 *jamesii*	-- 2------2---------- ------- ----- - 00000000 2000 00 000
31 *ramsayi*	-- 2------2---------- ------- ----- - 00000000 2000 00 000
32 *maculipalpis*	-- 2------2---------- ------- ----- - 02000000 2200 20 002
33 *pretoriensis*	-- 2------2---------- ------- ----- - 02000000 2200 20 002

0 and 2 = homozygous condition for the two alternative conditions; 1 = heterozygous condition; - = unknown.

Table 2. Characters and hypothetical character states for non-terminal clades of the Myzomyia + Neocellia cladogram (Fig. 1).

Characters			Clades														
			34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	Xa	(Subbarao et al. 1988)	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
1	Xb	(Subbarao et al. 1988)	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2*	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	2	0	2	2	2
3	2h	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
4	2i	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
5	2i[1]	(Subbarao et al. 1988)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
6	2j	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
7	2k	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
8	2l	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
9	2m	(Green 1982)	0	2	0	0	2	2	0	0	2	0	2	2	2	2	2
10	2n	(Green 1982)	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
11	2o	(Green 1982)	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
12	2p	(Green 1982)	0	2	0	0	2	2	0	0	2	0	0	0	0	0	0
13	2q	(Green 1982)	0	2	0	0	2	2	0	0	2	0	0	0	0	0	0
14	2r	(Green 1982)	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0
15	2s	(Green 1982)	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0
16	2t	(Green 1982)	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
17	2u	(Green 1982)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	2v	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
19	2w	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
20	2x	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
21	3c	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
22	3d	(Green 1982)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	3h	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
24	3i	(Green 1982)	2	0	2	2	0	0	2	0	0	0	0	0	0	0	0
25	3j	(Green 1982)	2	0	2	2	0	0	2	0	0	0	0	0	0	0	0
26	3k	(Green 1982)	0	2	0	0	2	2	0	0	2	0	0	0	0	0	0
27	3l	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
28	3o	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
29	4a	(Green 1982)	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
30	4b	(Green 1982)	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
31	4e	(Green 1982)	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
32	4j	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
33	4k	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
34	4l	(Green 1982)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
35	Xa	(Green et al. 1985)	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2
36	2e	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0
37	2f	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
38	2l	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	2r	(Green and Baimai 1984)	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
40	2k	(Green and Baimai 1984)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
41	2j	(Green and Baimai 1984)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
42	2o	(Green and Baimai 1984)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
43	2n	(Green and Baimai 1984)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
44	3a	(Green et al. 1985)	-	0	-	-	-	0	-	-	-	-	2	-	2	2	2
45	3d	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0

Table 2 continues.

Table 2. Continued.

Characters			Clades													
			34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
46	3k	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	3l	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	4s	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
49	4x	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0
50	5c	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0
51	5d	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0
52	5e	(Green et al. 1985)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0

"-" means "either 0 or 2". Character names are taken from their original source (indicated in brackets), except for 2*, which is the unnamed terminal inversion mentioned by Green et al. (1985). Note that "identical" names may be applied to different characters, e.g., characters 22 and 45. Characters are numbered starting from 0, following the format for Hennig86 version 1.5.

homologous (i.e., standard) segment if it is encompassed within another inversion. Thus, the breakpoints are of interest, not the orientation *per se*. In order to recognize the presence of an "inversion," the full segment (i.e., both breakpoints) has to be recognized, while recognition of the "standard" (i.e., absence of the alternative condition) may be confirmed through presence (or recognition) of just one of the "breakpoints."

Table 3. *Anopheles gambiae* complex, character matrix.

Anopheles	Characters
0 *subpictus*	-- 00 - -
1 *arabiensis*	22 11 2 2
2 *bwambae*	22 00 0 0
3 *gambiae*	00 11 1 2
4 *melas*	22 00 0 0
5 *merus*	00 00 0 2
6 *quadriannulatus*	22 00 0 2

Scorings are taken from the original sources indicated in Table 4. 0 and 2 = homozygous condition for the two alternative conditions; 1 = heterozygous condition; - = unknown.

The cladistic approach to reconstruction of phylogenies is based on grouping according to shared derived features or synapomorphic character states (Hennig 1966, Wiley 1981, Farris 1982a); and that some states are derived relative to others rests on the logic that character states are additive rather than discrete entities (Platnick 1979). One of the main problems is to deduce the direction of these transformation series, i.e., which character states are derived (apomorphic) and which are ancestral (plesiomorphic). In a cladistic context, this can be approached in three ways: by outgroup comparison, by the discovery of fossil series, and by ontogenetic studies (Wiley 1981, Farris 1982a). For chromosome inversion data, only the outgroup comparison is

Table 4. Characters and hypothetical character states for non-terminal clades of the consensus cladogram for the Gambiae Complex (Fig. 2C).

Characters		Clades 7 8 9
0 Xa	(Coluzzi et al. 1979; G.B. White 1973, 1985)	2 0 2
1 Xg	(Coluzzi et al. 1979; G.B. White 1973, 1985)	2 0 2
2 2b	(Coluzzi et al. 1979; G.B. White 1973, 1985)	0 0 0
3 2bc	(Coluzzi et al. 1979; G.B. White 1973, 1985)	0 0 0
4 3a	(Coluzzi et al. 1979; G.B. White 1973, 1985)	0 0 0
5 5a	(Coluzzi et al. 1979; G.B. White 1973, 1985)	0 2 2

Character names are taken from their original source (indicated in brackets). 0 and 2 = homozygous condition for the two alternative conditions; 1 = heterozygous condition.

feasible. Green (1982), citing Yates et al. (1979), explicitly wanted to determine the relative derivedness of chromosome inversion data "by observing the overall patterns, plus outgroup comparisons." This was further elaborated by Green et al. (1985), who stated that "the alternative which is common within the group is probably ancestral" and "that alternative which is shared with a group of species outside the one under study is probably ancestral." The former, however, is a misapplication, like considering wings in hexapods as ancestral just because they are very common. Note that "common" differs and is distinct from "widespread" (Farris 1982b). To assess how widespread (not necessarily common!) an inversion is, we need either outgroup information for the inversion or phylogenetically informative data from other characters for which outgroup data is available. Green et al. (1985) stated that outgroup comparison and relative commonness "are not independent criteria since both simply depend on relative commonness of one alternative over the other either within the group under study, or within this group and groups closely related to it . . . When these two criteria give contradictory answers, e.g., an alternative is rare within the group but also occurs as the only alternative in the outgroup, then the latter criterion [= outgroup comparison] is considered the more important indication." In this way, they reduce the application of "common-equals-primitive" to cases where outgroup comparison is inapplicable, for example because chromosomal homologies have not been recognized. In the character matrices of Green (1982:Fig. 7) and Green et al. (1985:Fig. 6), the designation of relative plesiomorphy and apomorphy have been included without explicitly indicating which decisions were based on outgroup comparison, which could be done by scoring the outgroup along with the ingroup. As a consequence, the data cannot produce consistent cladograms. Only through outgroup comparison will it be possible to postulate transformation series, that are by their nature hypothetical constructs. It is a misconception when Green (1982) stated that: "logically [outgroup comparison] must fall away as more interseries homologies are discovered and *Cellia* as a whole becomes the group." Widening the ingroup to encompass species previously used as outgroup will immediately create the need for another outgroup. Adding more data, e.g., through new information on inversion homologies, will not in itself change our primary hypothesis of out- versus ingroups. Only when another distribution of taxa seems to provide a more probable explanation for the distribution of character states (i.e., with fewer *ad hoc* hypotheses to explain homoplasies) will rearrangements have to be done. In the present case, only few interserial homologies are known between Myzomyia and Neocellia, but we can still assemble all species in one matrix (Table 1) and leave character states for which our knowledge is insufficient as "unknown" (scored as "-"). Analyzing the matrix requires that one or more taxa are chosen as outgroup because this is the only way we can root our transformation series.

Cladogram A of Green (1982) for Myzomyia is difficult to evaluate as he does not use the data to fix a position for *An. demeilloni* Evans. He indicated that inversion **m** is plesiomorphic contrary to the scheme in his Fig. 7. Green's (1982) cladogram B is in accordance with the data presented, although the plesiomorphic nature of inversion **n** is not explained. Also, assuming that the ancestor to the entire Myzomyia Series, except *An. demeilloni*, was polymorphic with regard to inversions **n** and **m** may not be the most parsimonious solution. The present consensus cladogram (Fig. 1) agrees with both cladograms proposed by Green (1982) in the recognition of the major species groups, namely: 1) *An. funestus* Giles, *An. vaneedeni*, *An. parensis* Gillies, *An. confusus* Evans and Leeson; 2) *An. rivulorum* Leeson, *An. fuscivenosus* Leeson; 3) *An. leesoni* Evans, *An. fluviatilis* James, Culicifacies Complex; 4) *An. wellcomei* Theobald, *An. theileri* Edwards; and 5) *An. demeilloni*. It also corroborates the placement of the Culicifacies Complex as more closely related to *An. fluviatilis* and *An. leesoni* than to any other species or species group included. Revision of the formal definition of the Funestus Group (see, e.g., Gillies and De Meillon 1968) is recommended.

There is agreement in the species-level phylogenetic relationships within these species groups, except that the present analysis gives *An. parensis* and *An. confusus* as equally related to the clade *An. funestus* + *An. vaneedeni*.

In the present consensus cladogram of the Myzomyia and Neocellia Series (Fig. 1), the Wellcomei Group, from which *An. wellcomei* and *An. theileri* were scored, is given as the sister group of the Funestus Group + the Rivulorum Group, corroborated by the shared possession of inversion **m** and thus differing from both alternatives given by Green (1982). This has reduced what Green (1982) described as a "total discontinuity between the *funestus/rivulorum*-groups and the other groups" to uncertainty with regard to the position of the Demeilloni Group within the Myzomyia Series. Obviously, more knowledge on chromosome configurations within the Demeilloni Group is required, along with a better resolution of chromosomal homologies to the outgroup.

For the Neocellia Series, Green et al. (1985) considered presence of inversion **3a** as indicative of inclusion within the series; i.e., they considered **3a** as apomorphic at this level. But as they did not consider *An. stephensi* Liston, which possesses the alternative **3+^{a}**, as the sister group to all other members of the Neocellia Series, they had to assume a polymorphism in the common ancestor and repeated, parallel fixations. When all Neocellia members are considered as outgroup, the analysis cannot designate which of the alternatives **3a/3+^{a}** most probably is apomorphic; i.e., the available chromosome inversion data does not exclude the possibility that the Neocellia Series is paraphyletic with regard to the Myzomyia Series.

Green et al. (1985) defined three species-groups among the species of the Neocellia Series examined: 1) *An. rufipes* (Gough), *An. maculipalpis* Giles, *An. pretoriensis* (Theobald); 2) *An. pulcherrimus* Theobald, *An. annularis* Wulp, *An. philippinensis* Ludlow, *An. nivipes* (Theobald); and 3) *An. superpictus* Grassi, *An. stephensi*, Maculatus Complex. The present analysis has confirmed only group 1) consisting of the three Afrotropical species. It may be noted that this group cannot be "reinforced by the extrinsic data from their geographical distribution" (Green et al. 1985). Their confinement to the African continent cannot *a priori* be taken as evidence of phylogenetic relationship. This group is recognized because it alone possesses unique shared inversions, for which outgroup data is present and for which no conflicting evidence makes other possibilities equally probable. The conflicting evidence presented by inversions **2e** and **3a** (Green et al. 1985:Fig. 6) implies either reversals or a polymorphic ancestor, and therefore more than one equally probable evolutionary scenario. Therefore, the present data does not provide arguments for the recognition of groups 2) and 3) of above as monophyletic groups.

In the Gambiae Complex of the Pyretophorus Series, we will find an almost complete lack of outgroup information, and the resolution of phylogenetic relationships is reduced accordingly. In the phylogenetic hypotheses put forward by Coluzzi et al. (1979) and G.B. White (1973, 1985), *An. quadriannulatus* (Theobald) is considered a basal or even a stem lineage; thus, Coluzzi et al. (1979) stated: "the chromosomal relationships point to the zoophilic and exophilic *An. quadriannulatus* as an intermediate or (more probably) ancestral phylogenetic step." The notion of extant ancestors, however, is conceptually somewhat dubious as the reproductive isolation (which is the main reason for treating *An. bwambae* White and *An. quadriannulatus* as distinct species) means that every member has a unique evolutionary branch. However, Coluzzi et al. (1979) and G.B. White (1973, 1985) did not consider the entire chromosomal configuration of *An. quadriannulatus* as ancestral, as the two floating inversions "**Xf**" and "**2Ri**" were scored as unique to this species and apparently considered as derived.

White (1985) considered the karyotype of *An. quadriannulatus* plesiomorphic because of the almost entirely zoophilic habits of this species and its patchy, relict distribution (Gillies and Coetzee 1987:Fig. 23). Zoophily and exophily are probably under genetic control and at least partly correlated to the pattern of chromosome inversions (Coluzzi et al. 1979).

They may therefore be treated as character states and evaluated by outgroup comparison. Unfortunately, knowledge of chromosomal configurations for close relatives of the Gambiae Complex is indeed meager, but according to White (1980) "The closest known chromosomal relative of the *gambiae* complex is the Oriental *A. subpictus*." *Anopheles subpictus* is a species complex of at least two species (Suguna 1982, Subbarao et al. 1988) containing vectors of both malaria and Bancroftian filariasis (White 1989a,b). It may be at least as parsimonious to consider the zoophilic habit of *An. quadriannulatus*, and therefore its non-vector status, as derived relative to the remaining members of the Gambiae Complex. Therefore, we cannot consider zoophily and exophily in the Gambiae Complex as evidence of a basal position in the cladogram.

The distributional patchiness is evidence of a previously more widespread distribution, or of dispersal. Although a minimum age for a given species may be hypothesized from the geological and/or paleoclimatic history for the area in combination with present knowledge of autecology, the distribution *per se* cannot be taken as evidence of phylogenetic relationships. Admittedly, the distribution of *An. quadriannulatus*, *An. gambiae* Giles and *An. arabiensis* could easily be interpreted in the way that the former has been replaced by the two latter species in the areas between its current distributional foci, but this cannot in itself be taken as evidence that *An. quadriannulatus* possesses an ancestral chromosomal pattern and certainly not that *An. quadriannulatus* is the sister group of all other members of the Gambiae Complex. Many other scenarios could be put forward. Again, the only rigorous test for transformation series polarity is that of outgroup comparison.

The three cladograms in Fig. 2 result in a Nelson consensus cladogram, which equals and thus corroborates the original hypothesis of Coluzzi et al. (1979) and G.B. White (1973, 1985). The most important "result" emerging from the three cladograms is that we are far from an understanding of the evolution of the Gambiae Complex. However, the cladograms enable us to propose a solution for the apparent conflict in the chromosomal differences between *An. arabiensis* and *An. gambiae* on the one hand, and the striking phenotypic similarities on the other. Rather than evolutionary convergence (Coluzzi et al. 1979), the similarities may be explained as symplesiomorphies if *An. arabiensis* is the closest relative of the clade *An. gambiae* + *An. merus* Dönitz. The major question is how to root the transformation series **3+^{a}-3a**, **X+^{a}-Xa** and **X+^{g}-Xg** as we cannot *a priori* assume the "standard" to be ancestral.

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