Morphological characterization of the genital armature of male and female hybrids from crosses between *Glossina palpalis palpalis* and *Glossina palpalis gambiensis* (Diptera: Glossinidae)

M.J.B. Vreysen [1 *] A. M.V. Van der Vloedt [2 **]

Key words

Glossina palpalis palpalis - *Glossina palpalis gambiensis* - Hybrid - Morphology - Genital system.

Summary

The subspecies *Glossina palpalis palpalis* (*Gpp*) originating from Nigeria and *Glossina palpalis gambiensis* (*Gpg*) originating from Burkina Faso could be separated morphometrically based upon the width of the terminal dilatations of the male inferior claspers. Intermediate values were obtained for male hybrids, but the average size of the head of the parameres was significantly determined by maternal descendance, i.e. the average width of the head of the inferior claspers of male hybrids from *Gpg* x *Gpp* cross was significantly larger than that of hybrids from the reciprocal cross. Morphological characters of the inferior claspers of the male hybrids were distinctly different depending on the cross. The dorsal plates of the genital armature of female *Gpg* were significantly longer but less wide than those of female *Gpp*. Both subspecies could be separated with a minimal overlap (7%) by plotting the length of the dorsal plates against their width. Female hybrids of *Gpp* x *Gpg* cross had dorsal plates which were significantly longer and wider than those of hybrids from the reciprocal cross.

■ INTRODUCTION

The two closely related subspecies *Glossina palpalis palpalis* (Robineau-Desvoidy) (*Gpp*) and *Glossina palpalis gambiensis* Vanderplank (*Gpg*) are the predominant vectors of animal and human trypanosomosis in West-Africa (1), where they are found in distinct geographic regions. *Gpp* inhabits the forest galleries along the riverbanks in the humid savannah and forests, whereas *Gpg* is confined to the river systems of the dry and humid savannah (3). Although both subspecies hybridize readily under laboratory conditions without any inclination for assortative mating (13), there is no overlap in their distribution (2, 3). These subspecies seem to occur sympatrically only in a very narrow zone in Côte d'Ivoire, where they interact with each other (3, 7, 9). The male F_1 sterility is considered to be the mechanism responsible for this narrow band of hybridization (3, 7). The taxonomic status of *Gpp* and *Gpg* as two distinct subspecies was ascertained by Vanderplank (12), based upon results from crossbreeding experiments and from the analysis of the male fly parameres. This was later confirmed by Machado (8). In the zone of Côte d'Ivoire where these populations interact, a few specimens have been collected with morphological characteristics intermediate between those of the two subspecies (3, 7). It has been assumed that these specimens were the offspring of a successful cross mating between the two subspecies, but these assumptions have never been substantiated by a more in depth analysis of the external genitalia of the hybrids. The data described in this paper contribute to a more detailed morphometrical characterization of the inferior claspers of male hybrids and the genital plates of female hybrids, descending from laboratory crosses of *Gpp* and *Gpg*.

■ MATERIALS AND METHODS

Flies

The *Gpp* and *Gpg* parental lines originated from Kaduna (Nigeria) and Bobo-Dioulasso (Burkina Faso), respectively. The hybrid flies were derived from crossbreeding experiments as described by Vreysen and Van der Vloedt (13). In all cross designations, maternal lines are given first, paternal lines second.

Preparation of the inferior claspers and genital plates

Permanent preparations were made of the inferior claspers of the males of the parental lines and of the resulting hybrids. The

1. Entomology Unit, Joint FAO/IAEA Programme, International Atomic Energy Agency's Laboratories, 2444 Seibersdorf, Austria

2. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Insect and Pest Control Section, International Atomic Energy Agency, PO Box 100, Vienna, Austria

* Correspondence: IAEA Project RAF/5/040, c/o Ethiopian Science and Technology Commission, PO Box 19917, Addis Ababa, Ethiopia

Tel.: 215 1 51 67 70; Fax: 251 1 51 66 58; E-mail: estc@telecom.net.et

** Deceased

complete hypopygium was removed from the male flies' abdomen and cleared overnight in warm 10% potassium hydroxide and then transferred for 5-10 min to glacial 100% acetic acid. The inferior claspers were removed from the hypopygium under binocular and mounted in Euparal. Measurements of the terminal dilatations of the inferior claspers were made under a Leitz compound microscope at x 64 magnification. For each male, the average value of both inferior claspers was used for the analysis. The number of macrotrichae or bristles on the body of the inferior claspers was counted on photographs taken with the Leitz compound microscope. The same technique was used to make permanent preparations of the external genitals, i.e. the dorsal and anal plates of female *Gpg* and *Gpp* and female hybrids resulting from the crosses (8). Maceration in warm 10% potassium hydroxide was limited to 15-30 min to prevent the dorsal and anal plates from becoming completely translucent. Measurements were made of the length and width of the dorsal plates and the length of the anal plates. The average value of the left and right dorsal and anal plates of each female was used in the analysis.

Statistical analysis

Data on the measurements of the genital armature of male and female flies were analyzed by analysis of variance. In case significant differences were observed, the means were separated by Tuckey's HSD test at $P < 0.05$ (10).

■ RESULTS

Male genital armature

Biometrical analysis

The frequency distribution of the different size classes of the width of the terminal dilatations of the inferior claspers (TDIC) of *Gpp*, *Gpg* and their hybrids is presented in figure 1. The distribution of size classes of both subspecies TDIC was completely separated. A small overlap was observed between the various size classes of male *Gpp* and the hybrids from *Gpg* x *Gpp* cross (n = 2, 1.2%) and between those of male *Gpg* and the hybrids from *Gpp* x *Gpg* cross (n = 1, 1.0%). The mean width of the TDIC varied significantly between the parental lines and their hybrids ($F_{3,255}$ = 762.1, $P < 0.0001$) and was significantly larger in male *Gpg* than in male *Gpp* (Tuckey's HSD, $P < 0.05$). Intermediate values were observed for the hybrids but the mean width of the TDIC of hybrids from *Gpg* x *Gpp* cross was significantly larger than that of hybrids from the reciprocal cross ($P < 0.05$). The distributions of size classes of the TDIC of both parental lines and their hybrids were slightly negatively skewed, and there was a tendency towards a leptokurtosis in *Gpg* and hybrids from *Gpp* x *Gpg* cross. However, none of the distributions departed significantly from normality ($P > 0.05$). The number of macrotrichae or apical bristles on the TDIC was limited to 2 or 3 in 88.7% of the examined *Gpp* males, but 86% of the *Gpg* males had 3 or 4 apical bristles. Most of the hybrids had 3-4 macrotrichae on the TDIC (83.9% in male *Gpp* x *Gpg* and 87.8% in male *Gpg* x *Gpp*). The mean number of macrotrichae on the body of the inferior claspers (between 55.9 and 56.9) was similar for both parental lines and their hybrids ($F_{3,184}$ = 0.191, P = 0.90).

Morphological aspects

The general morphology of the inferior claspers of the *Gpp* and *Gpg* parental lines used in the laboratory crosses is shown in figure 2. The inferior clasper body of *Gpp* of Nigerian origin was characterized by (i) parallel internal and external borderlines, (ii) a neck which has the same width over its entire length, (iii) an internal border of the body making a straight line, (iv) the absence of an internal hunk, but a pronounced external hunk with tangents making an angle of approximately 90°, (v) narrow terminal dilatations with the internal lobe very little pronounced and the external lobe varying from a round structure to a larger hammer-like character.

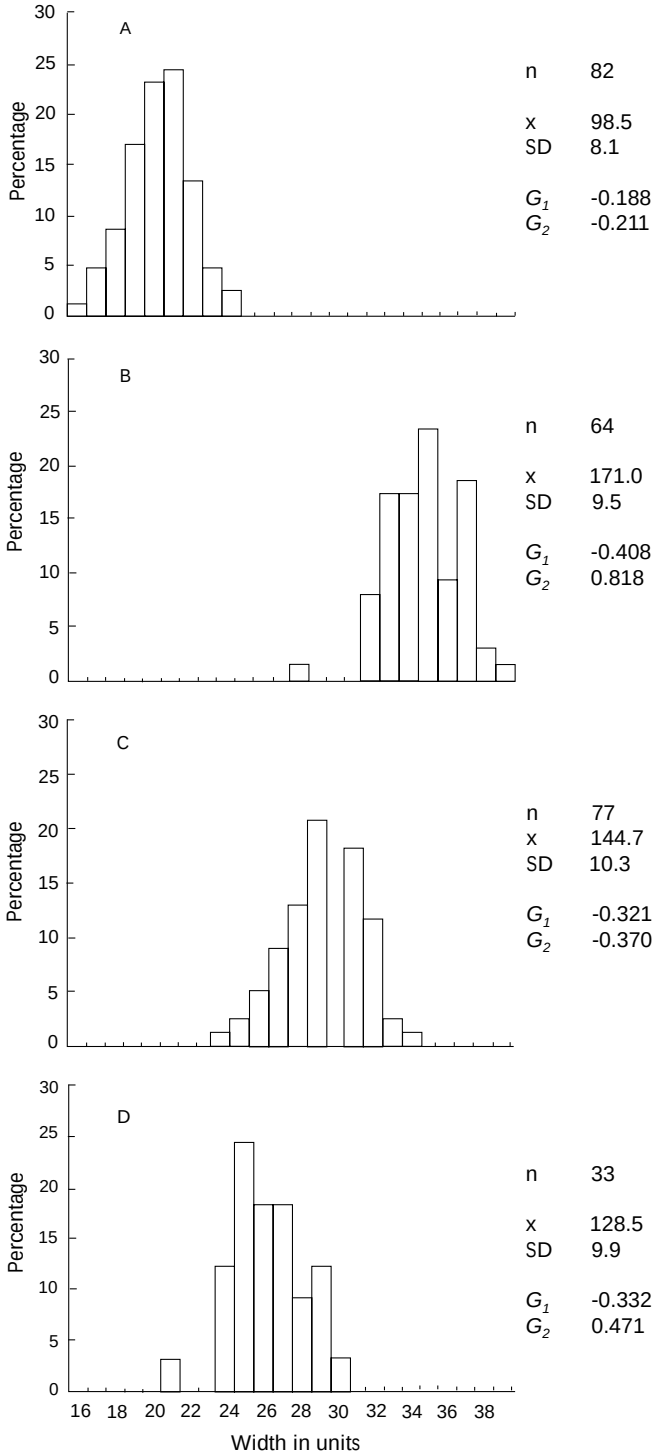

***Figure 1**: Frequency distribution of the different size classes of the width of the terminal dilatation of the inferior claspers of male (A)* Glossina palpalis palpalis *(*Gpp*), (B)* Glossina palpalis gambiensis *(*Gpg*), (C) hybrids from* Gpg *x* Gpp *cross and (D) hybrids from* Gpp *x* Gpg *cross (1 unit is 5 µm, n = sample size, x is mean (in µm), SD = standard deviation, G_1 = skewness, G_2 = kurtosis).*

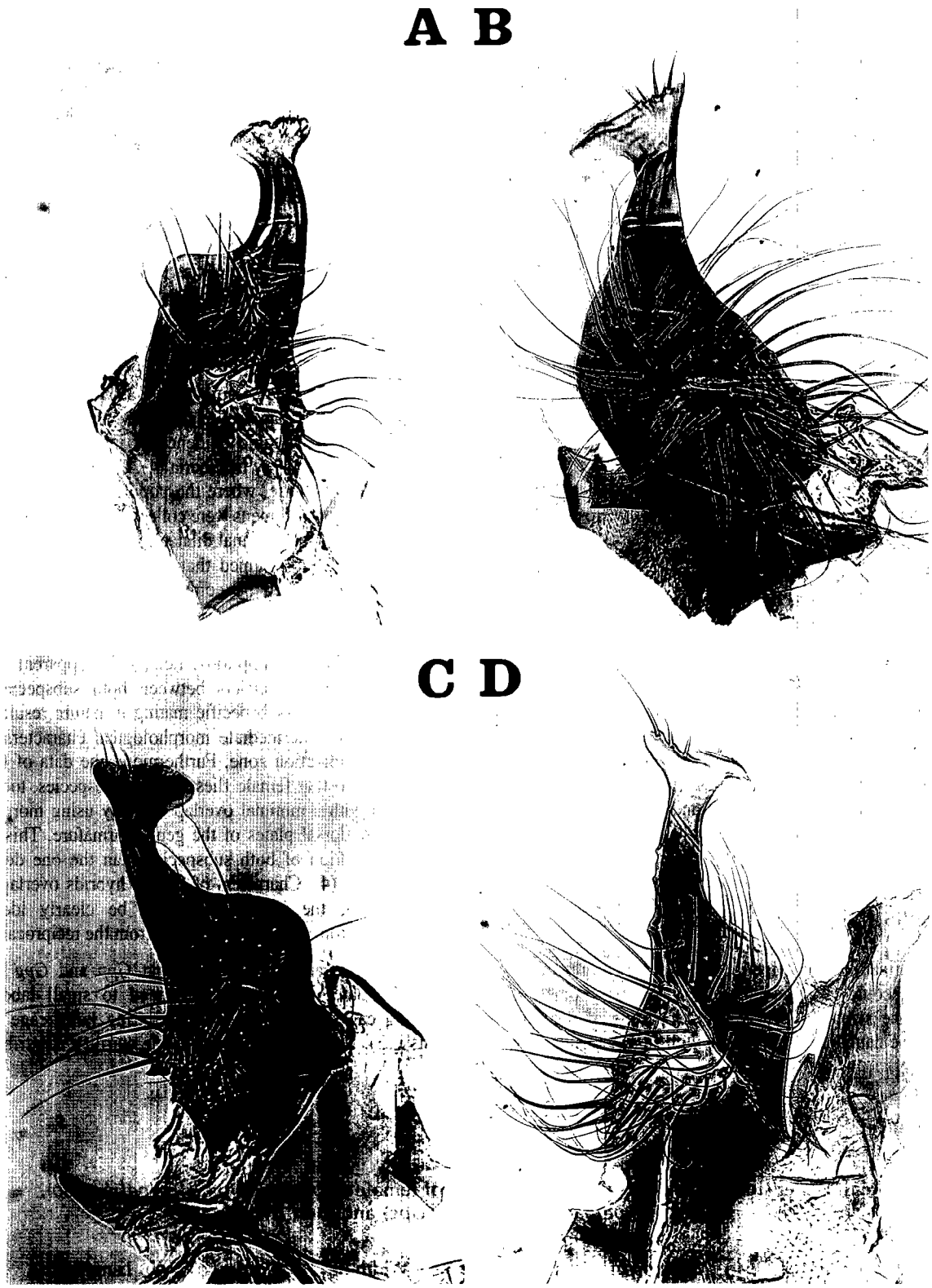

***Figure 2**: Diagrams of the inferior claspers of male (A)* Glossina palpalis palpalis *(Gpp), (B)* Glossina palpalis gambiensis *(Gpg), (C) hybrids from* Gpp *x* Gpg *cross and (D) hybrids from* Gpg *x* Gpp *cross.*

The inferior clasper body of *Gpg* of Burkina Faso origin was characterized by (i) the inner side of the body being bent externally, resulting in non-parallel internal and external borderlines, (ii) a clasper neck narrowing gradually towards the terminal dilatation making the external hunk less prominent as in *Gpp*, (iii) a small internal hunk apparent at the base of the body and (iv) large terminal dilatations with a very pronounced external lobe.

Marked differences were observed in the general morphological appearance of the inferior claspers of the hybrids depending on the cross. The inferior claspers of male hybrids from *Gpp* x *Gpg* cross were characterized by (i) the internal and external lobes of the terminal dilatations equal in size, (ii) an internal border only slightly bent, (iii) a very prominent external hunk, with at the base a depression or internal indentation, (iv) an indentation in the

terminal dilatation, present but less prominent, as in the hybrids from the *Gpg* x *Gpp* cross (figure 2C). Hybrid males from the reciprocal *Gpg* x *Gpp* cross had inferior claspers with (i) a hammer-like terminal dilatation with the external lobe larger than the internal lobe, (ii) a bent internal borderline (with less inclination as in *Gpp*) resulting in an internal hunk, (iii) a prominent external hunk (more prominent as in *Gpg*), but with considerable variation in size, (iv) an indentation present at the distal end of the terminal dilatation (not present in *Gpg*) (figure 2D).

Biometry of the female genital armature

Female *Gpg* displayed dorsal plates, which were on average significantly longer and significantly less wide than those of *Gpp* (table I). The dorsal plates of female hybrids from *Gpp* x *Gpg* cross were significantly longer but had a width similar to those of female *Gpp*, whereas female hybrids from *Gpg* x *Gpp* cross had dorsal plates which were significantly less long but had a width similar to those of the parental *Gpg*. Analysis of the length of the anal plates showed significant differences between the two subspecies, between *Gpp* and female hybrids from the two crosses and between female *Gpg* and females resulting from *Gpg* x *Gpp* cross.

Frequency distributions of the length of the dorsal and anal plates overlapped considerably for both parental lines and their hybrids (figure 3). Both subspecies could be separated for 93% by plotting the length of the dorsal plates against their width (figure 4). Characters overlapped more (18.7%) in females resulting from crosses.

The length and width of the dorsal plates were significantly correlated in female *Gpp*, in female *Gpp* x *Gpg* and in female *Gpg* x *Gpp* but not in female *Gpg* (table II). There was no correlation between the length of the dorsal plates and the length of the anal plates in female *Gpp*, in female *Gpg* and in female *Gpp* x *Gpg*. Both parameters were, however, positively correlated in females of the *Gpg* x *Gpp* cross.

■ DISCUSSION

Male *Gpp* originating from Nigeria and male *Gpg* originating from Burkina Faso could be completely separated morphometrically, based upon the size of the terminal dilatations of the inferior claspers. These data are in accordance with survey results of West Africa carried out to outline the geographical distribution of both subspecies (2, 3, 4, 7, 9). In addition, male hybrids of both crosses showed intermediate values for the size of the head of the parameres, but significant differences were obtained between hybrids of both crosses depending on the maternal descendance. This study also revealed that the general morphology of the inferior claspers of male hybrids depends on the cross.

In the past, the occurrence of hybrids in nature had remained an enigmatic issue due to the lack of a detailed description of hybrid morphology. Machado alludes to the existence of specimens with intermediate morphological characters in Liberia, Ghana and Côte d'Ivoire and considers the entire South of the latter as a large zone of hybridization (8). These findings are questioned by Gouteux and Dagnogo who point out the existence of considerable morphological variation in the size of the head of the inferior claspers of male flies sampled in the Southern part of the Côte d'Ivoire (6): their statistical analysis reveals a homogeneous *Gpp* population. Likewise, no evidence of intergradation was found during a detailed survey in Liberia (4). Only in the narrow contact zone in Côte d'Ivoire, where the populations of both subspecies converge, a few specimens were collected with intermediate values for the width of the terminal dilatations of the inferior claspers (3, 7, 9). It has been assumed that these specimens were hybrids, which was confirmed by comparison with only 11 specimens resulting from laboratory crosses (7). The present more extensive study can now confirm these observations and corroborate previously made assumptions, i.e. (i) the apparent absence of ethological mating barriers between both subspecies, (ii) the occurrence of inter-subspecific mating in nature resulting in (iii) offspring with intermediate morphological characters in a very narrow hybridization zone. Furthermore, the data of the present study showed that female flies of both subspecies, too, could be separated with a minimal overlap (7%) by using morphometrical data of the dorsal plates of the genital armature. This allowed a better separation of both subspecies than the one described by Garms *et al.* (4). Characters of female hybrids overlapped more, but 80% of the individuals could be clearly identified as descending from *Gpp* x *Gpg* cross or from the reciprocal cross.

Laboratory studies have shown that *Gpp* and *Gpg* males and females mate readily when confined to small laboratory fly holding cages (5, 7, 11, 13) and during large cage laboratory tests (13). The resulting hybrid male sterility is attributed to an

Table I

Mean dimensions of the dorsal and anal plates of female *Glossina palpalis palpalis* (*Gpp*), *Glossina palpalis gambiensis* (*Gpg*) and their hybrids

Female	Male	Num. of females	Length of dorsal plates (µm)		F-ratio [df*]	Width of dorsal plates (µm)		F-ratio [df*]	Length of anal plates (µm)		F-ratio [df*]
			Mean	SD		Mean	SD		Mean	SD	
Gpp	*Gpp*	74	362.8 [a]	22.4	99.9***	340.3 [a]	20.1	21.4***	248.5 [a]	14.4	43.3***
Gpg	*Gpg*	69	427.2 [b]	24.4	[3,203]	321.2 [b]	19.7	[3,203]	280.4 [b]	19.0	[3,203]
Gpp	*Gpg*	30	420.9 [b]	21.2		346.5 [a]	22.5		275.7 [b]	21.9	
Gpg	*Gpp*	32	374.6 [a]	31.5		313.6 [b]	26.5		258.3 [c]	16.6	

[a, b] Means not followed by the same letter are significantly different (Tuckey's HSD, $P < 0.05$)
* Degrees of freedom
*** $P < 0.001$

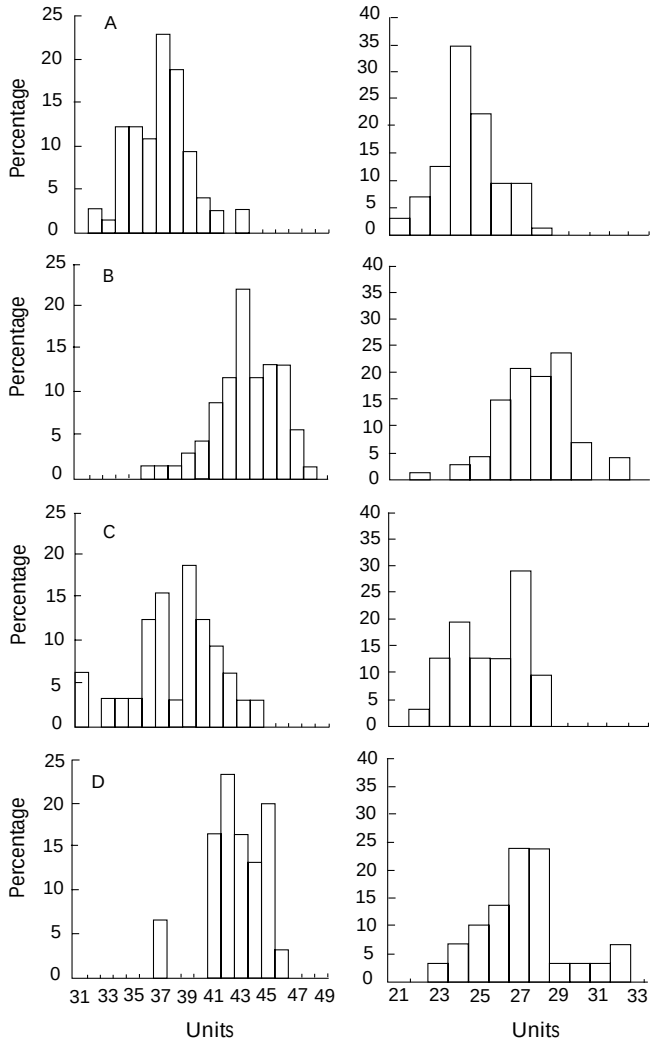

***Figure 3**: Frequency distribution of the different size classes of the length of the dorsal (left) and anal (right) plates of female (A)* Glossina palpalis palpalis *(*Gpp*), (B)* Glossina palpalis gambiensis *(*Gpg*), (C) hybrids from* Gpg *x* Gpp *cross and (D) hybrids from* Gpp *x* Gpg *cross (1 unit is 10 µm).*

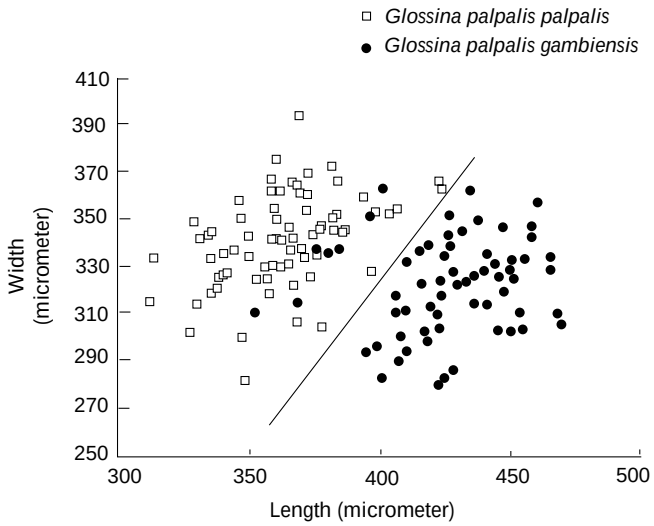

***Figure 4**: Length of the dorsal plates plotted against their width.*

Table II

Pearson's correlation coefficient between the length and width of the dorsal plates and the length of the dorsal and anal plates of female *Gpp*, *Gpg* and their hybrids

	Length and width of dorsal plates	Length of dorsal and anal plates
Gpp	0.4369 **	0.2262 n.s.
Gpg	0.1069 n.s.	0.0150 n.s.
Gpp x *Gpg*	0.4389 *	0.1427 n.s.
Gpg x *Gpp*	0.5979 ***	0.5543 **

* P < 0.05; ** P < 0.01; *** P < 0.001; n.s. = not significant

interruption of the spermatogenesis process (11) and to the low or complete absence of motility of sparsely produced mature hybrid sperm (13). The field and laboratory data accumulated to date indicate the potential of using these subspecies for control purposes based on male hybrid sterility (13). In addition, as the mating behavior of both *Gpp* and *Gpg* is not altered by a sterilizing radiation treatment (13), gamma sterilized males of one subspecies could be used to eradicate target populations of both subspecies.

The morphological characterization of male and female hybrids resulting from *Gpp* and *Gpg* crosses described here confirms the hypothesis that male and female hybrids exhibit intermediate morphological characters. The hybrid morphology described in this paper is, however, derived from hybrids descending from parental lines of two distinct geographical areas (Nigeria and Burkina Faso). It would be worthwhile extending this study to analyze the reproductive parameters and to morphometrically characterize the hybrids from subspecies originating from two neighboring localities.

Acknowledgments

The authors would like to thank Dr. J.C. van Lenteren and Dr. W. Takken (Wageningen Agricultural University), and Dr. U.H. Feldmann (IAEA) for reviewing the manuscript.

REFERENCES

1. BUXTON P.A., 1955. The natural history of tsetse flies. An account of the biology of the genus *Glossina* (Diptera). London, UK, Lewis HK, 816 p. (Mem. London Sch. Hyg. trop. Med. No. 10)

2. CHALLIER A., DEJARDIN J., 1987. Variations morphologiques chez les mâles de *Glossina palpalis palpalis* (Rob.-Desv.) et *G. p. gambiensis* Vanderplank. Leurs implications taxinomiques. *Cah. Orstom, Sér. Ent. méd. Parasitol.*, n° spécial : 83-99.

3. CHALLIER A., GOUTEUX J.-P., COOSEMANS M., 1983. La limite géographique entre les sous-espèces *Glossina palpalis palpalis* (Rob.-Desv.) et *G. palpalis gambiensis* Vanderplank (Diptera : Glossinidae) en Afrique occidentale. *Cah. Orstom, Sér. Ent. méd. Parasitol.*, **21**: 207-220.

4. GARMS R., MEHLITZ D., ZILLMANN U., 1987. Geographical distribution of *Glossina palpalis gambiensis* and *Glossina palpalis palpalis* in Liberia. *Med. vet. Ent.*, **1**: 343-347.

5. GOODING R.H., 1988. Preliminary analysis of genetics of hybrid sterility in crosses of *Glossina palpalis palpalis* (Robineau-Desvoidy) and *Glossina palpalis gambiensis* Vanderplank. *Can. Ent.*, **120**: 997-1001.

6. GOUTEUX J.-P., DAGNOGO M., 1985. Homogénéité morphologique des genitalia mâles de *Glossina palpalis palpalis* (Diptera : Muscidae) en Côte d'Ivoire. *Cah. Orstom, Sér. Ent. méd. Parasitol.*, **23** : 55-59.

7. GOUTEUX J.-P., MILLET P., 1984. Observations sur le contact entre *Glossina palpalis palpalis* (Rob.-Desv.,1830) et *Glossina palpalis gambiensis* Vanderplank, 1949, dans la région de Bouaké (Côte d'Ivoire). *Tropenmed. Parasit.*, **35** : 157-159.

8. MACHADO de BARROS A., 1954. Révision systématique de glossines du groupe *palpalis* (Diptera). *Publ. Cul. co. diam. Angola*, **22** : 189 p.

9. NEKPENI E.B., DAGNOGO M., EOUZAN J.P., 1989. Détermination de la limite géographique entre deux sous-espèces de glossines en Côte d'Ivoire : *Glossina palpalis palpalis* (Robineau-Desvoidy, 1830) et *Glossina palpalis gambiensis* Vanderplank, 1949. *Trop. Med. Parasit.*, **40** : 12-15.

10. SOKAL R.R., ROHLF F.J., 1995. Biometry, 3rd ed. New York, NY, USA, WH Freeman, 887 p.

11. SOUTHERN D.I., 1980. Chromosome diversity in tsetse flies. In: Blackman R.L., Hewitt, G.M., Ashburner M. Eds., Insect cytogenetics (Symposia of the Royal Entomological Society of London, No. 10). Oxford, UK, Blackwell's, p. 225-243.

12. VANDERPLANK F.L., 1949. The classification of *Glossina palpalis* including the description of a new subspecies and hybrids. *Proc. R. ent. Soc. London (b)*, **18**: 69-77.

13. VREYSEN M.J.B., VAN DER VLOEDT A.M.V., 1990. The effect of intersubspecific hybridization and gamma radiation on the reproductive biology of *Glossina palpalis palpalis* (Robineau-Desvoidy) and *Glossina palpalis gambiensis* Vanderplank. *Ann. Soc. belge Méd. trop.*, **70**: 145-158.

Reçu le 19.1.99, accepté le 12.3.99

Résumé

Vreysen M.J.B., Van der Vloedt A. M.V. Caractérisation morphologique de l'armature génitale des hybrides mâles et femelles issus de croisements entre *Glossina palpalis palpalis* et *Glossina palpalis gambiensis* (Diptera : Glossinidae)

Les sous-espèces *Glossina palpalis palpalis* (*Gpp*) provenant du Nigeria et *G. p. gambiensis* (*Gpg*) provenant du Burkina Faso pouvaient être différenciées par des caractères morphologiques, en se basant, pour les mâles, sur la largeur des dilatations terminales des forcipules inférieurs. Des valeurs intermédiaires ont été mesurées chez les hybrides mâles, mais la dimension moyenne de la tête des paramères était déterminée, significativement, par la descendance maternelle. Ainsi, la largeur moyenne de la tête des forcipules inférieurs des hybrides mâles issus du croisement *Gpg* x *Gpp* était plus grande, d'une manière significative, que celle des hybrides issus du croisement inverse. Les caractères morphologiques des forcipules inférieurs des hybrides mâles montraient des différences nettes en fonction du croisement. Les plaques dorsales de l'armature génitale des femelles *Gpg* étaient plus longues mais moins larges, de manière significative, que celles des femelles *Gpp*. Les deux sous-espèces pouvaient être séparées avec un chevauchement minimal (7 p. 100) en reportant la longueur des plaques dorsales sur l'axe des abscisses et la largeur sur celui des ordonnées. Les plaques dorsales de l'armature génitale des hybrides femelles *Gpp* x *Gpg* étaient significativement plus longues et plus larges que celles des hybrides du croisement inverse.

Mots-clés : *Glossina palpalis palpalis* - *Glossina palpalis gambiensis* - Hybride - Morphologie - Système génital.

Resumen

Vreysen M.J.B., Van der Vloedt A. M.V. Caracterización morfológica del armazón genital de los machos y hembras híbridos de cruces entre *Glossina palpalis palpalis* y *Glossina palpalis gambiensis* (Diptera: Glossinidae)

Las subespecies *Glossina palpalis palpalis* (*Gpp*), originaria de Nigeria, y *Glossina palpalis gambiensis* (*Gpg*), originaria de Burkina Faso, pueden separarse morfometricamente en base al ancho de las dilataciones terminales de los apéndices copulatorios inferiores de los machos. Se obtuvieron valores intermedios para los machos híbridos, pero el tamaño medio de la cabeza de los parámeros fue significativamente determinado por descencia materna, es decir el ancho medio de la cabeza de los apéndices copulatorios inferiores de los machos híbridos del cruce *Gpg* x *Gpp* fue significativamente más largo que el de los híbridos de los cruces recíprocos. Los caracteres morfológicos de los apéndices copulatorios inferiores de los machos híbridos fueron diferentes según el cruce. Las placas dorsales de la armazón genital de la *Gpg* hembra fueron significativamente más largos, pero menos anchos, cuando comparados con los de la hembra *Gpp*. Ambas subespecies pueden diferenciarse con un traslapo mínimo (7%), mediante la esquematización del largo de las placas dorsales contra el ancho. Las hembras híbridas de cruces *Gpp* x *Gpg* presentaron placas dorsales significativamente más largas y anchas que las de cruces recíprocos.

Palabras clave: *Glossina palpalis palpalis* - *Glossina palpalis gambiensis* - Híbrido - Morfología - Aparato genital.