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The Water Balance of Tsetse Pupae

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THE WATER BALANCE OF TSETSE PUPAE

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[Plate 10]

CONTENTS

1. The pupal period of *Glossina morsitans* can be divided into a number of different stages on the basis of anatomical changes:

(*a*) The 3rd instar larva which comprises the first day of pupal development. During this time water is lost at relatively high rates in part by transpiration through the puparium and in part through the supernumerary stigmata of the polypneustic lobes.

(*b*) The 4th instar larva which comprises the next 3 days; rates of transpiration are somewhat lower due to the deposition inside the puparium of a second integument. But water is lost by excretion as well as by transpiration; this excretory loss is regulated so that the more water is lost by transpiration the less is excreted.

(*c*) The pupa and pharate adult. The larva-pupa moult occurs on about the 5th day and a few days later the hypodermis retracts from the pupal skin and the adult cuticle begins to be laid down. Following the formation of the very impermeable pupal skin there is a substantial decrease in the rate of transpiration. At this stage water is lost also through the pupal spiracle, but this loss is under active control, the rate being a function of the water content of the pupa.

(*d*) A few days before emergence the pupal skin dries out and its permeability increases; spiracular control ceases at the same time, so that rates of water loss during this period are high.

2. The regulation of excretory loss and of spiracular loss, combined with extremely low membrane permeabilities, confers a high degree of resistance to desiccation on the pupa of *G. morsitans*; it can complete its development at humidities as low as 10 % r.h.

3. It is shown that in areas where a prolonged dry season occurs the humidity to which pupae are subject under natural conditions may fall to about 40 % r.h.; if the pupal sites are exposed to the action of grass fibres even lower humidities may be experienced.

4. There is a close correlation between the resistance to desiccation of the pupae of different species of tsetse fly and the habitat in which they occur, and it is suggested that the water balance of the pupa may have been an important limiting factor in the invasion of semi-arid and arid habitats.

5. The development of resistance to desiccation in tsetse pupae has involved a decrease in the permeability of puparium and pupal skin, a decrease in the size of respiratory lobes and an increase in the percentage loss of water which can be tolerated before development stops.

Introduction

The water balance of tsetse pupae has been studied by a number of workers, and in broad outline there is substantial agreement between results from different laboratories (Buxton & Lewis 1934; Mellanby 1936; Jack 1939; Willett 1953). But the results of field investigations show no such agreement and their interpretation is consequently a matter of some difficulty. For example, Nash (1939) and Chorley (1929) have noted a seasonal cycle in the mortality of pupae under more or less natural conditions, and have related these fluctuations to correlated changes in certain physical factors. But if the arguments are examined in the light of laboratory studies it appears far from certain that the physical factors could in detail be responsible for the observed effects. Other workers have, on the contrary, been impressed with the constant emergence rate of pupae collected from the field at different seasons; some have attributed this constancy to constancy of the pupal environment (Buxton & Lewis 1934; Mellanby 1936); while others have suggested that the pupa is so well fitted to withstand adverse conditions that seasonal fluctuations in temperature and humidity would be unlikely to affect the mortality (Jack 1939). Although this divergence of views may in some measure be attributed to differences between the species examined, it seemed that the whole subject would merit re-investigation, with particular reference to the integration of field and laboratory data.

The results will be presented in three parts; the first dealing with the properties and processes affecting water balance in the pupa of *Glossina morsitans* Westwood; the second relating these findings to conditions obtaining in the habitat of this species; and the third comparing the water balance of a number of different species.

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PART I. THE WATER BALANCE OF *GLOSSINA MORSITANS* WESTWOOD

Material and methods

Pupae were bred from a population of *G. morsitans* females maintained in the laboratory; larvipositions usually occurred in the late afternoon and early evening, but some larvae were deposited during the night. The breeding tubes were not cleared until eight o'clock in the morning so that the pupae used for experiments were anything from 1 to 18 hr old at the start. This was a complication which could not readily be overcome, apart from the inconvenience attending regular collection during the evening and night. The breeding of tsetse flies at Shinyanga is still a somewhat precarious undertaking; more than a third of the total number of larvipositions were abortions which failed to pupate, and to attain even this degree of success we have reason to believe that disturbance of the females must be kept at a minimum. So hourly inspection of the breeding tubes, which would be necessary to enable the age of pupae to be accurately determined, was precluded by the nature of the experimental material.

For determination of weight loss pupae were exposed singly on perforated zinc trays in desiccators and weighed at intervals on an analytical balance which could be read to 0·1 mg. A later series of experiments were done with a Mettler balance weighing to 0·01 mg.

Humidities were controlled with appropriate mixtures of potassium hydroxide and water (Buxton & Mellanby 1934) and with calcium chloride. Most of the work was carried out at a time when there was no constant-temperature room at Shinyanga. Experiments were done in an inner room where diurnal fluctuations in temperature were negligible (less than 0·3° C); but the temperature showed a considerable seasonal shift with a maximum of about 29° C and a minimum of about 24° C. For purposes of the present publication data at temperatures outside the range of 24·2 to 25·8° C have been excluded. In 1956 a constant-temperature room became available and, to confirm previous findings, a series of experiments was carried out at $24{\cdot}7 \pm 0{\cdot}3°$ C using the more sensitive balance; from this are derived the data given in figures 1, 2, 3, 5, 6 and 8, and tables 1 and 2.

Loss of water by transpiration is most accurately expressed in terms of surface area (see p. 195); but weight is also lost by excretion and this loss cannot reasonably be related to surface. For the sake of simplicity both transpiratory and excretory loss will be expressed as mg % of the original weight; since differences in mean size between different samples of pupae were slight the error introduced by ignoring the surface effect would be negligible.

The adsorption of water vapour by puparial shells was determined: taking the water content in dry air as 0 %, the percentage regain follows a hyperbolic curve typical of hygroscopic materials in general; with a % water content of 5·5 at 60 % r.h., 9·5 at 80 % r.h. rising sharply to 16·0 at 98 % r.h. The loss of adsorbed water from fully hydrated puparial shells is complete in 24 h (98 % complete in 7 h), so when pupae were moved from one humidity to another this period was allowed for equilibration before weight loss determination began. In some experiments such a period of equilibration was precluded (e.g. figure 4), in which case the weight loss recorded after a change from one humidity to another was corrected on the basis of the resorption curve of empty shells.

In what follows, weight loss will be considered synonymous with water loss. Fat (i.e. ether soluble substances) is the only solid which can be shown to be consumed in the course of development (see table 3, p. 190); and since the oxidation of 1 mg fat produces 1·07 mg water its consumption will not significantly influence the gross weight of the pupa.

Results

(1) *The course of development*

The 3rd instar larva of the tsetse fly is deposited on the surface of the soil and burrows to a depth usually of 1 to 6 cm before pupating. At 25° C the duration of pupal development, from the time that the larval skin hardens and darkens to form the puparium till the emergence of the adult fly, is about 30 days. This period can be subdivided into a number of stages on the basis of anatomical changes (see also figure 9, p. 193).

(*a*) *The 3rd instar puparium* (*day* 0 *to day* 1)

This represents the quiescent phase of the 3rd instar with the larval cuticle sclerotized to form the puparial shell.

(*b*) *The 4th instar larva* (*day* 2 *to day* 4)

The larva is retracted from the inner surface of the puparium and is limited by a tenuous integument representing the cuticle of the 4th instar. The polypneustic lobes have been evacuated, except for remnants of the 3rd instar tracheal apparatus (Bursell 1955), and the tracheal trunks of the 3rd instar enter the larval body through two posterior spiracles. These spiracles can be shown to be functional by way of the 3rd instar tracheal trunks, for if the lobes of the puparium are immersed in coloured paraffin oil under reduced pressure, and the pressure is subsequently released, dissection shows that the paraffin penetrates through the supernumerary stigmata into the 3rd instar tracheal trunks and thence to the tracheal system of the 4th instar.

Connexion with the puparium is also maintained at the hind gut which opens to the surface of the puparium by way of the 3rd instar anus.

(*c*) *The pupa and pharate adult* (*day* 5 *to day* 30)

On about the 5th day the 4th instar larva moults and its exuvium can be recovered in the form of two pale rolls of skin adhering to the lateral walls of the puparial shell.* The imaginal buds of the larva are evaginated at this time and the thin pupal skin laid down. Between the hypodermis and the pupal skin there is secreted a quantity of clear fluid below which the cuticle of the adult is formed. This fluid is gradually resorbed in the course of further development, until at a day or two before emergence no liquid remains and the pupal skin dries out.

The pupa has a single pair of functional spiracles, as demonstrated by the uptake of liquid paraffin under reduced pressure; they open antero-laterally on the thorax in the position to be occupied by the prothoracic spiracles of the adult.

To avoid continual circumlocution in the account which follows, the word puparium will be used to denote the puparial shell and its contents, regardless of whether this be the

* I am indebted to Mr D. L. Johns for first drawing my attention to this feature.

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3rd instar, 4th instar, pupa or pharate adult. The term 'pupal period', denoting the interval between hardening and darkening of the 3rd instar larva and the emergence of the adult, has the sanction of long usage and will be retained, despite the fact that pupation *sensu strictu* does not occur until 5 days after puparium formation.

(2) *Weight loss at different stages of development*

Figure 1 shows the rate of water loss in dry air at different stages of development. The larval instars are characterized by high rates of loss; after pupation the rate of transpiration falls rapidly to a minimum on about the 9th day. There is subsequently a gradual increase until at a day or two before emergence the rate of water loss rises rapidly to equal the early values.

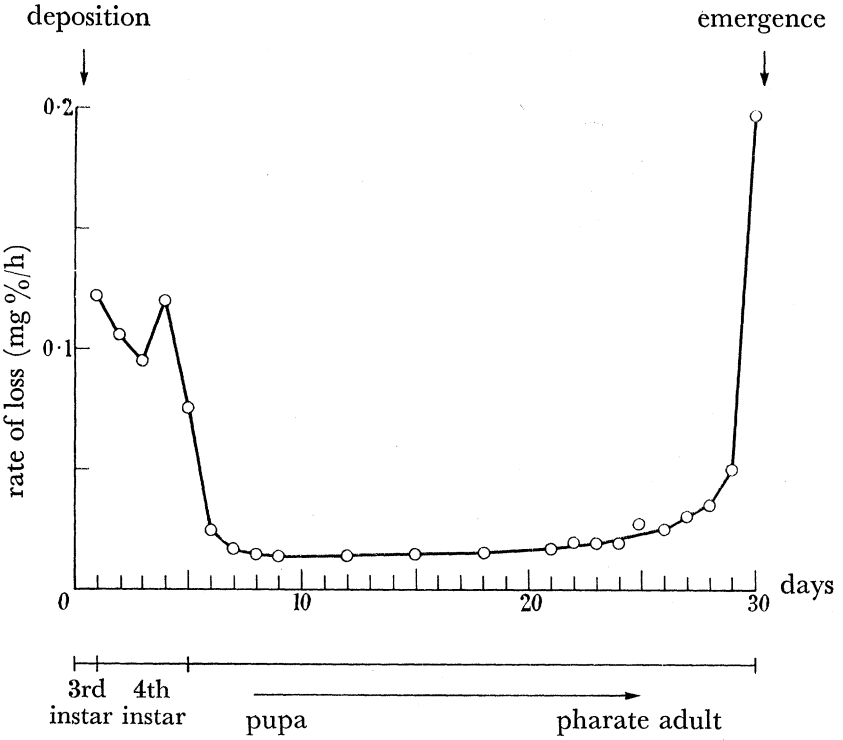

FIGURE 1

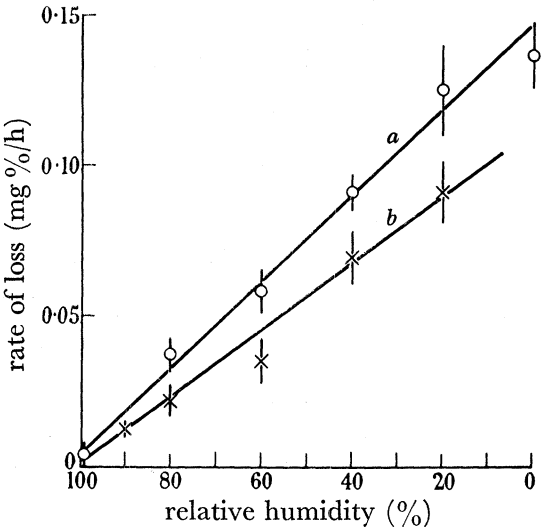

FIGURE 2

FIGURE 1. The rate of water loss in dry air during the course of development of *G. morsitans*.

FIGURE 2. The rate of water loss on the first (*a*) and last (*b*) day of pupal life as a function of relative humidity. In this and other figures the fiducial limits are represented by a vertical line passing through the mean.

To obtain information about the mechanisms which underlie these changes in transpiration rate, the relation between humidity and the rate of loss was studied for each of the developmental stages.

(*a*) *The 3rd instar puparium*

Figure 2*a* shows the effect of humidity on the rate of loss in weight during the first day after puparium formation. The relation is clearly rectilinear and extrapolates to zero loss in saturated air. In other words, weight loss is directly proportional to the saturation deficiency of the atmosphere and may be presumed to be caused by transpiration alone.

(*b*) *The 4th instar larva*

The rate of loss of the 4th instar larva is plotted as a function of humidity in figure 3 I, curve *a*; here there is obviously no simple relation between weight loss and saturation deficiency. The loss is entirely independent of humidity between 100 and 40 % r.h.; only at still lower values does the rate of loss increase as the atmosphere gets drier.

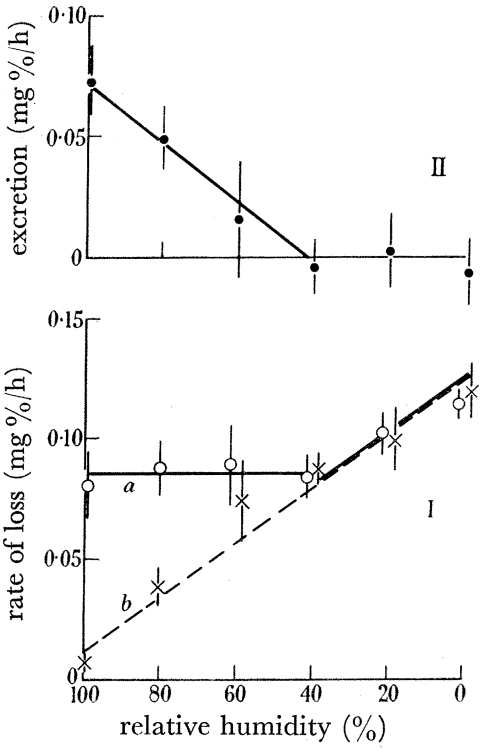

FIGURE 3

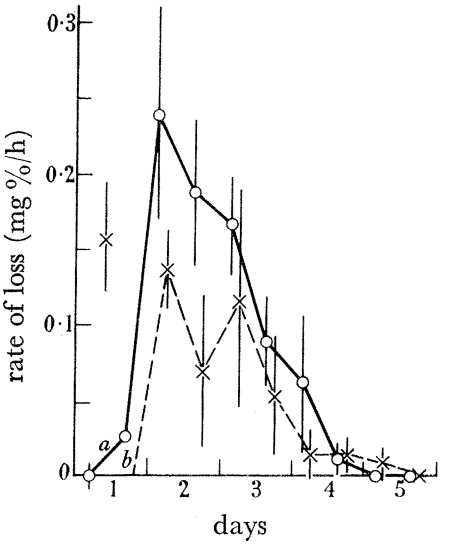

FIGURE 4

FIGURE 3. I. The rate of water loss of the 4th instar larva at different humidities. (*a*) normal puparia, (*b*) puparia with anal block. II. The rate of excretion of the 4th instar larva as a function of humidity (see text). For the sake of clarity in this and other figures, where fiducial limits overlap for observations made at the same humidity the values have been horizontally staggered.

FIGURE 4. The rate of water loss at 98 % r.h. during the 4th larval instar. (*a*) puparia maintained at 98 % r.h. throughout, (*b*) puparia maintained at 0 % r.h. on the first day (the rate of transpiration on first day has been included in the figure).

The high rates of loss recorded in saturated air must be ascribed to some process other than transpiration. In view of the observations of Edwards (1954) who described an exudation of fluid from the pupal anus of *Musca*, the most likely alternative seemed to be excretion; and a series of experiments was carried out to determine the rate of loss of puparia in which the anus has been blocked with paraffin wax and excretory loss thus prevented. The results are plotted in figure 3 I, curve *b*. The relation between humidity and weight loss with puparia treated in this manner does not depart significantly from rectilinearity; it extrapolates close to zero thus confirming that the high rates of loss of normal puparia in saturated air are due to excretion.

It has not been possible to observe the actual outflow of excretory fluid in *Glossina*, perhaps because the rate of excretion is much lower than in *Musca* (about 0·025 mg/h as

compared with 0·12 mg/h). Nor does the exudate of tsetse puparia leave an obvious deposit after the water has evaporated off, which may be why the phenomenon has escaped attention for so long.

By comparing the curve for normal puparia with that of puparia whose excretion has been prevented, it is possible to get an idea of the weight loss through excretion during the 4th instar larval period (see figure 3 II). It is clear that excretion is maximal in saturated air and decreases steadily in lower humidities until at 40 % r.h. the amount of weight lost by excretion is too small to be detected by present methods.

This effect of humidity on the rate of excretion appears to be an indirect one, based on differences in water content of the 4th instar larvae. For if this is reduced by desiccation of the 3rd instar, the rate of excretion during the 4th instar, as determined by weight loss in saturated air, is markedly decreased. Figure 4, curve *a*, represents the course of excretion when the 3rd instar puparium has been maintained in 98 % r.h.; curve *b* shows the reduced rates when the 3rd instar puparium has been desiccated for 24 h in 0 % r.h.

The total amount excreted is $8{\cdot}85 \pm 0{\cdot}58$ mg % in group *a* and only $3{\cdot}66 \pm 0{\cdot}34$ mg % in group *b* ($t = 7{\cdot}74$, $P < 0{\cdot}001$); but if the water lost by transpiration on the first day is included in the group *b* loss, there is no significant difference between the two amounts ($8{\cdot}85 \pm 0{\cdot}58$ as compared with $7{\cdot}50 \pm 0{\cdot}31$ mg %; $t = 1{\cdot}95$; $P = 0{\cdot}1$ to $0{\cdot}05$).

The process of excretion in the 4th instar thus represents a mechanism for the regulation of water content; excessive loss of water by transpiration is compensated by a reduction in the amount of water excreted. Ideally this would mean that at the time of pupation the water content of pupae should be the same irrespective of the conditions to which they have been subjected. But it has been shown in figure 3 that at humidities below 40 % r.h. more water is lost by transpiration than is normally excreted, compensation is incomplete and the water content is reduced to subnormal levels.

(*c*) *The pupa*

(i) *Transpiration after deposition of the pupal skin.* Figure 5, curve *b*, shows the rate of transpiration of pupae which have been maintained at different humidities since puparium formation. The curve shows a marked departure from rectilinearity, the loss at low humidities being relatively small. If puparia are kept in 98 % r.h. until the pupal moult and then exposed to a range of humidities, the rate of water loss is a linear function of humidity as shown in curve *a*. It seems, therefore, that exposure of the larval instars to low humidities causes a reduction in the rate of transpiration of the pupa.

In order to determine more precisely the nature of this effect, batches of puparia were maintained at different humidities for 8 days after puparium formation. They were then exposed for 24 h to 0 % r.h. to allow the puparial shell to come into equilibrium with dry air, after which the loss in weight during 10 days following was measured. In figure 6 the rate of loss during this time has been plotted as a function of the humidity to which the larval instars were subjected. It is clear that the drier the air during larval instars the lower is the rate of transpiration of the pupa.

If the transpiration rates are plotted against the amount of water lost during the larval instars rather than against the humidity to which these have been subjected, a similar

rectilinear relation is obtained, although the fit is not quite so good.* But analysis of the data for individual humidities suggests that the amount of water lost, or alternatively the water content, may in fact be the causal link between the humidity experienced during early stages and the pupal transpiration rate. For in all but the highest humidities there is a negative correlation between the amount of water lost during the larval instars and the rate of transpiration of the pupa. Correlation coefficients were available for a

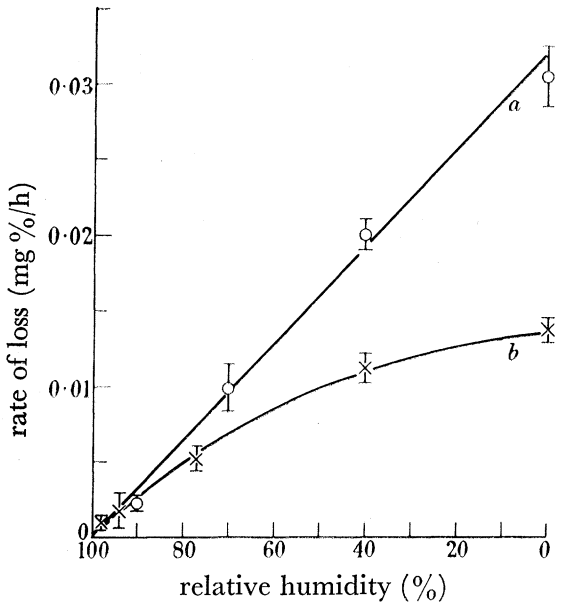

FIGURE 5

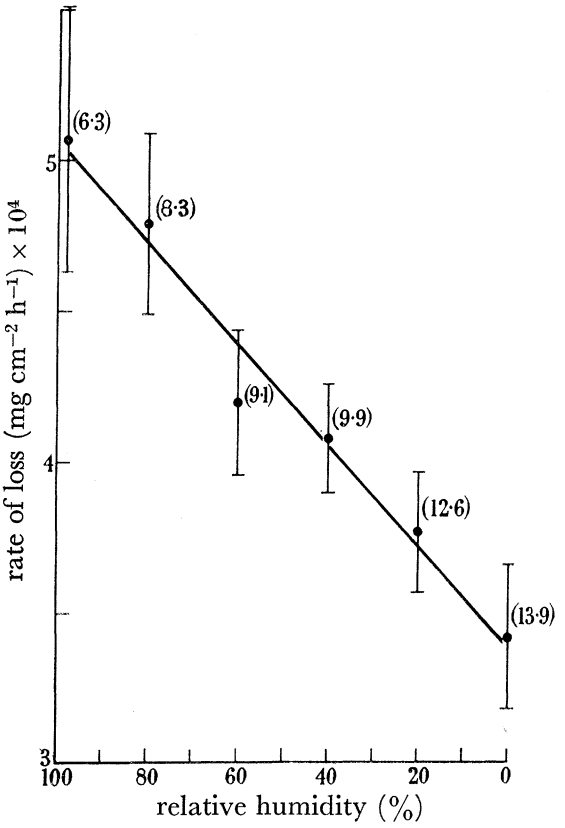

FIGURE 6

FIGURE 5. The rate of water loss of pupae at different relative humidities. (*a*) puparia maintained at 98 % r.h. for the first 8 days of the pupal period. (*b*) puparia maintained at the stated humidities throughout.

FIGURE 6. The rate of water loss of pupae in dry air as a function of the humidity to which puparia were exposed during the first 8 days of the pupal period. Figures in brackets give the amount of water lost during the first 8 days. Rates have been expressed in terms of surface area for comparison with pupae from the field (see Part II).

number of experiments at different humidities, and these were summed by z transformation. The mean z for humidities below 80 % r.h. was $-0{\cdot}401$ with $t=3{\cdot}33$ and $P=0{\cdot}01$. At higher humidities excretion predominates over transpiration, and excretion has been shown to be regulated according to the amount of water already lost. So the recorded differences in weight loss under these circumstances would reflect differences in the amount

* The fact that the amount of water lost is related to humidity over the whole range may seem at variance with the conclusions reached in the previous section. But the excretory regulation which renders weight loss in part independent of humidity extends only to about the 4th day (see figure 4). Transpiration rates are relatively high until the 6th day (figure 1) and it is the loss between the 4th and the 6th day which is in the main responsible for the recorded differences in the amount of water lost.

lost between puparium formation and recovery from the breeding tubes rather than be the cause of corresponding differences in the water content of pupa.* The loss from individual puparia would therefore not be related to the rate of transpiration, and in fact the value of z for humidities above 80 % r.h. is $+0{\cdot}003$ with $P = 0{\cdot}1$.

It appears then that the rate of transpiration of the pupa is a function of its water content so that the greater the water loss during early stages, the less the transpiration during the remainder of pupal life. The most likely site of this regulation is the pupal spiracle, and some experiments were carried out with carbon dioxide + air mixtures to determine

TABLE 1. THE EFFECT OF CARBON DIOXIDE ON THE RATE OF TRANSPIRATION OF PUPAE IN DRY AIR

relative humidity day 1 to 8	rate of loss (mg %/h) in air	N	in CO_2	N	difference	P
98 %	$0{\cdot}0287 \pm 0{\cdot}0012$	6	$0{\cdot}0446 \pm 0{\cdot}0048$	6	0·0159	0·01
0 %	$0{\cdot}0163 \pm 0{\cdot}0002$	6	$0{\cdot}0411 \pm 0{\cdot}0038$	6	0·0248	0·01
difference	0·0124	—	0·0035	—	—	—
P	0·01	—	0·6	—	—	—

N = number of pupae.

whether an effect analogous to the opening of adult spiracles with carbon dioxide could be observed with the pupa. The results are set out in table 1, and show that the rate of transpiration is doubled by exposure to 10 % carbon dioxide, and that in these circumstances there is no significant difference between puparia which have been maintained in 0 % r.h. since puparium formation, and puparia which have been maintained in 98 % r.h. This situation is very similar to that reported for the adult tsetse fly (Bursell 1957), and the results lend support to the view that in the pupa also the spiracles play a part in the regulation of transpiration.

An attempt was next made to test whether the increased water loss in 10 % carbon dioxide could in fact be attributed to relaxation of spiracular control. Difficulties were experienced in trying to expose the pupal spiracles without damage to the very tenuous pupal skin underlying the inner surface of the puparial shell—damage manifested in enormously increased rates of water loss. The procedure eventually adopted was to expose the puparia to 0 % r.h. for the first 8 to 10 days after puparium formation; this treatment causes extensive cracking of the puparial shell in a fairly high proportion of individuals. With these cracked specimens small fragments of the shell overlying the pupal spiracles could sometimes be peeled off without damage to the pupal skin.

Blocking of the pupal spiracles with 'Duco' lacquer caused a slight and doubtfully significant reduction in the rate of transpiration in air (see table 2). But while exposure to 10 % carbon dioxide caused more than a twofold increase in the rate of transpiration of pupae with free spiracles, it had no effect on pupae whose spiracles had been blocked. The somewhat high rates of transpiration of these pupae as compared with those in table 1 may resonably be ascribed to the removal of part of the puparial shell and extensive cracking of its remaining surface. This would leave the pupal skin as the sole barrier to

* This interpretation receives some support from the finding that the variance of water content at the time of recovery from the breeding tubes is significantly greater than at the time of the pupal moult for pupae maintained at 98 % r.h. (see table 3, day 0 and day 8).

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transpiration, whereas in normal pupae the puparial shell itself would contribute to the low permeability of the whole.

These experiments show fairly conclusively that the observed regulation of the transpiration rate of normal pupae may be attributed to some kind of spiracular regulation. How this regulation is effected is still a matter for conjecture. The structure of the pupal spiracle and its relation to the prothoracic spiracle of the developing adult is shown in

TABLE 2. THE EFFECT OF CARBON DIOXIDE ON THE RATE OF TRANSPIRATION IN DRY AIR OF PUPAE WHOSE SPIRACLES HAVE BEEN EXPOSED BY REMOVAL OF PARTS OF THE PUPARIAL SHELL

	rate of loss (mg %/h)					
spiracles	in air	N	in CO_2	N	difference	P
free	$0{\cdot}036 \pm 0{\cdot}005$	7	$0{\cdot}089 \pm 0{\cdot}007$	5	0·053	0·01
blocked	$0{\cdot}025 \pm 0{\cdot}002$	7	$0{\cdot}027 \pm 0{\cdot}004$	5	0·002	0·8
difference	0·011	—	0·062	—	—	—
P	0·05	—	0·01	—	—	—

N = number of pupae.

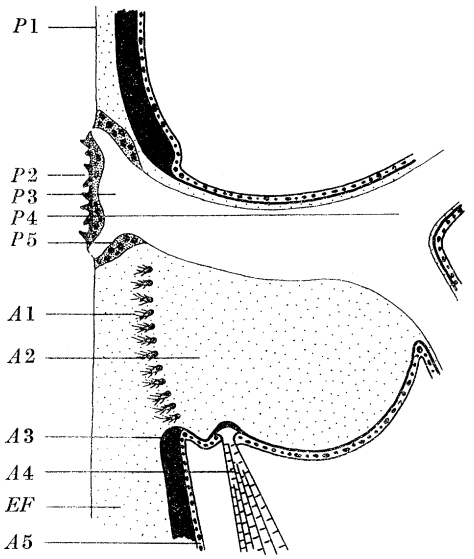

FIGURE 7. The pupal spiracle. $P1$, pupal skin; $P2$, pupal spiracle; $P3$, pupal air chamber; $P4$, pupal tracheae; $P5$, spiracular cells; $A1$, spiracular filter of adult; $A2$, adult airchamber; $A3$, adult cuticle; $A4$, spiracular muscle; $A5$, adult hypodermis; EF, ecdysial fluid.

figure 7. The tracheal system of the pupa communicates with the exterior through five or six minute pores which open along the perimeter of a small spiracular plate, the latter beset with short and blunt cuticular processes. The spiracular pores open into a corresponding number of air tubes, which converge to open into an air chamber lying centrally beneath the spiracular plate. This air chamber is continuous with the main tracheal trunk which subsequently divides into dorsal and ventral, anterior and posterior branches. Investing the air tubes from their origin at the air chamber to their opening at the surface of the spiracular plate are several layers of glandular cells (see figure 15, plate 10). It has not been possible to detect any ducts, intra- or extracellular, in connexion with these cells, nor has any trace of secretory products been observed.

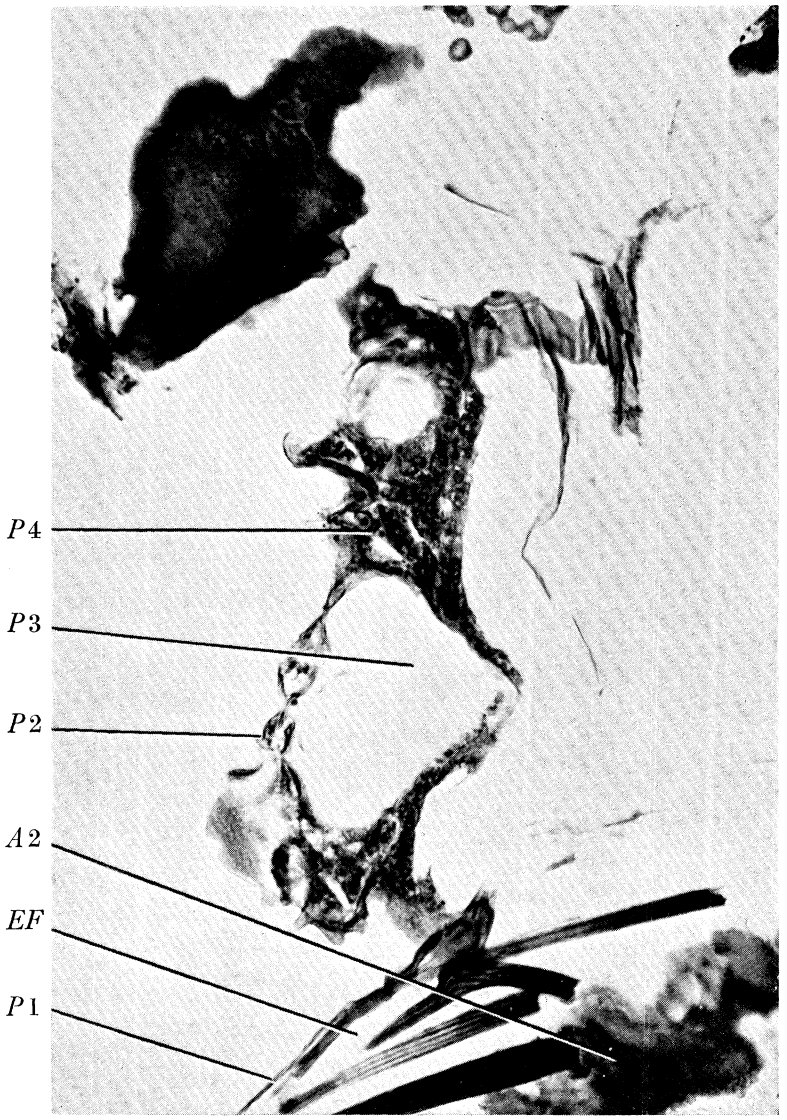

FIGURE 15. Section through the spiracle of pupa 23 days after puparium formation. *P*1, pupal skin; *P*2, pupal spiracle; *P*3, air chamber of pupal spiracle; *P*4, cells associated with pupal spiracle; *A*1, adult cuticle with spines; *EF*, ecdysial fluids.

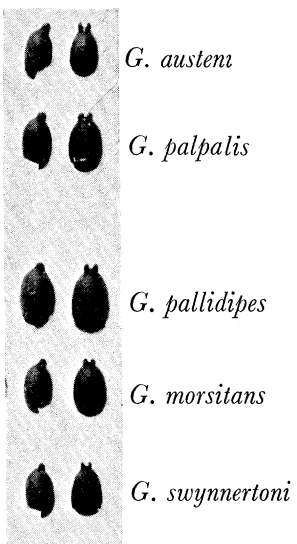

FIGURE 16. Puparia of some species of tsetse fly illustrating differences in the relative size of polypneustic lobes.

(*Facing p.* 188)

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Retraction of the hypodermis from the pupal skin takes place on about the 7th day after puparium formation (about 2 days after pupation) in preparation for the secretion of the adult cuticle. The only cells which do not take part in this retraction are the ones associated with the pupal spiracle. These persist in an apparently healthy condition right till the end of the pupal period (see figure 15, plate 10); during all of this time they are separated from the rest of the living tissue by ecdysial fluid, which fills the space between pupal skin and adult cuticle. This recalls the situation reported by Hinton (1955) who showed that at the pupal-adult moult of *Lipsothrix* a number of epidermal cells remain associated with the pupal cuticle of the spiracular gills, in isolation from the living animal.

As shown in figure 7, the prothoracic spiracle of the adult is laid down around the pupal spiracle; the latter enters the adult air chamber dorsally between the two rows of spiracular hairs which guard the prothoracic spiracle; and at ecdysis the pupal tracheae are shed through this aperture. The adult spiracle has been described by Geigy & Huber (1952); it has a well-developed muscle (shown in figure 7) by the contraction of which the spiracle is closed and the loss of water from the adult tracheal system minimized (Bursell 1957). The pupal spiracle has no such muscle; indeed the tracheal wall becomes detached from the tissues of the pupa soon after pupation. Nor could spiracular regulation in the pupa be ascribed to a precocious action of the adult spiracular muscle engaging indirectly on the pupal spiracle. For at a time when spiracular regulation is at its height (as judged by the difference in transpiration rate in pupae maintained at 0 and 98% r.h. during the early stages) there is very little sign of tissue differentiation, and certainly nothing that could be described as functional muscle tissue associated with the spiracle. It is clear, therefore, that spiracular regulation of water loss in the pupa cannot be attributed to the activity of spiracular muscles. Possibly the spiracular cells are involved in the regulatory mechanism, but in what way they exert their effect has not been determined.

The occurrence of spiracular regulation in the tsetse pupa is of interest in view of recent work on the respiratory metabolism of diapausing pupae (Punt 1950; Buck & Keister 1955; Schneiderman & Williams 1955). It seems likely that the two processes, the control of respiration and the control of water loss, are very intimately associated, and it is hoped that a study of respiratory control may provide a more sensitive approach to the problem of the regulatory mechanism.

(ii) *Pre-emergence loss.* As shown in figure 1, the rate of loss increases very markedly a day or two before emergence. The effect is somewhat exaggerated in this figure, because pupae which have been maintained since puparium formation in 0% r.h. invariably show extensive cracking of the puparial shell, and have in consequence an abnormally high permeability. In figure 2, curve *b*, the rate of loss on the last day of pupal life is plotted against humidity; the value for 0% r.h. has been ignored in drawing the curve.

The relation is seen to be rectilinear at a level somewhat below that which characterizes the 3rd instar puparium. It extrapolates to zero loss in saturated air, indicating that weight loss is caused entirely by transpiration.

The level of permeability on the last day of development suggests that a breakdown of the pupal skin has occurred, leaving the puparial shell as the limiting membrane. Such a breakdown might be accounted for by actual rupture of the pupal skin, caused by slight movements of the fly inside the puparium. A number of attempts were made to

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record such movements, by resting a small stylus, attached to a recording lever, on the pupal skin through a window cut in the puparial shell. Records were made on a smoked drum, the system being such that displacements of 50 μ or more would be detectable. No such movements occurred until the fly actually emerged, so the above explanation had to be abandoned.

The problem was investigated further by dissection of pupae on the day before emergence was expected. The front end of the puparium was embedded in paraffin wax to facilitate removal of the shell; and animals were anaesthetized by a 1 h exposure to carbon dioxide in order to prevent struggling during the dissection.

It was invariably found that at this time the pupal skin appeared completely dry and tended to adhere to the puparial shell so that it was impossible to remove the latter without rupturing the thin pupal membrane. By contrast pupae dissected 4 to 5 days before emergence could be removed readily without visible damage to the pupal skin, the space between it and the adult cuticle being filled with ecdysial fluid.

TABLE 3. CHANGES IN THE WATER AND FAT CONTENTS OF PUPAE IN THE COURSE OF DEVELOPMENT

age of pupae (days)	relative humidity (%)	water content (% of fatless wet weight)	fat content (% of total dry weight)	residual dry weight (% of original weight)	N
0	—	75·02 ± 0·32	31·94 ± 0·48	22·38 ± 0·28	21
3	98	72·55 ± 0·23	30·09 ± 0·52	22·66 ± 0·16	11
5	98	72·58 ± 0·29	29·46 ± 0·56	22·88 ± 0·21	9
8	98	72·79 ± 0·19	27·80 ± 0·37	22·58 ± 0·15	21
24	98	73·54 ± 0·30	18·20 ± 1·56	22·76 ± 0·21	11
3	0	73·02 ± 0·25	30·89 ± 0·55	21·90 ± 0·23	12
8	0	68·35 ± 0·43	27·82 ± 0·49	23·13 ± 0·20	13
24	0	69·00 ± 0·29	14·63 ± 0·94	22·82 ± 0·18	16

N = number of pupae.

The resorption of ecdysial fluid was in fact found to be coincident with the increase in transpiration rate, and it is suggested that the drying up of the delicate pupal membrane may in itself be sufficient to account for the loss of waterproofing capacity. Dehydration of natural membranes had been shown to cause marked alteration of membrane structure (Richards & Korda 1948); and the permeability of dried insect cuticle is often considerably greater than that of the natural integument (compare, for example, values obtained for *Rhodnius* by Wigglesworth (1945) using the intact animal, and by Beament (1954) using dried cuticle).

The pre-emergence transpiration is unaffected by the humidity to which pupae have previously been exposed, which indicates that at this time there is no spiracular control.

(3) *The production of metabolic water*

Substantial amounts of water are produced by the oxidation of fat, and since fat appears to constitute the main food reserve of the tsetse pupa (Buxton & Lewis 1934) it was of interest to determine to what extent its oxidation would affect the water balance. Determinations were accordingly made of fat content (ether soluble substances), water content and residual dry weight puparia maintained at 0 and 98% r.h. The results are shown in table 3.

With puparia kept at 98 % r.h. there is a sharp drop in the water content during the first few days; this is accounted for by the excretion of the 4th instar larva, as described above. After the 4th day there is a slow increase in water content, amounting to about 1 % by the end of the pupal period. The fat content decreases during the post-excretory period from about 30 % to less than 20 % of the dry weight, and there can be little doubt that the increase in water content may be ascribed to the combustion of fat with consequent production of metabolic water. Jack (1939) has shown that there is no uptake of water by pupae exposed to saturated air, beyond what can be accounted for by the hygroscopic properties of the puparial shell. This has been confirmed during the present investigation. Ignoring loss of water by transpiration (which at 98 % r.h. would be less than 0·02 mg per pupa for the period in question) it can be calculated that the breakdown of 1·14 mg fat produced 1·02 mg water, which is slightly less than the expected value of 1:1·07 (Wigglesworth 1953). The significance of this difference cannot be established with the data available, but it is interesting that there is a slight increase in residual dry weight during the course of development. Combining the data for the two humidities the values are $22{\cdot}37 \pm 0{\cdot}15$ % for day 3 and $22{\cdot}81 \pm 0{\cdot}12$ % for day 24, with $t = 2{\cdot}53$ and $P = 0{\cdot}01$. This represents a gain of 0·13 mg. It is possible that though most of the fat is broken down to carbon dioxide and water some is incorporated, perhaps as lipoproteins, in intra- or extracellular structures, and is in this way rendered immune from the solvent action of ether.

In 0 % r.h. the water content decreases continuously until pupation; after this the production of metabolic water is able to compensate for the loss of water by transpiration so that there is no significant difference between the water content on day 8 and on day 24.

The fat content of pupae maintained at 0 % r.h. is somewhat lower than that of pupae kept at 98 % r.h.; but the difference is not statistically significant ($P = 0{\cdot}1$ to $0{\cdot}05$) and cannot be taken as evidence that production of metabolic water is increased in dry air to compensate for the increased loss of water.

(4) *The waterproofing membranes*

(*a*) *The 3rd instar larva*

During this stage water is lost partly through the puparial shell and partly through the supernumerary stigmata of the polypneustic lobes. The loss from the tracheal system is not subject to active control, for it is the same in live and dead pupae and it is not significantly affected by carbon dioxide. By comparing the rate of water loss of puparia whose lobes have been blocked with 'Duco' lacquer with that of untreated controls it is possible to partition the total loss between these two sites of loss.

In 0 % r.h. the rate of loss of normal pupae is 0·121 mg %/h and of pupae whose lobes have been blocked it is 0·074 mg %/h. In other words about 40 % of the total loss takes place by way of the polypneustic lobes. Loss through the puparial shell is at the rate of 0·002 mg $cm^{-2}\,h^{-1}(mm\,Hg)^{-1}$, a permeability which falls within the normal range for insect cuticles (Wigglesworth 1945).

The rate of transpiration in dry air at different temperatures is shown in figure 8, curve *a*; the curvature of the logarithmic plot shows transpiration to be temperature sensitive (see Holdgate & Seal 1956), and by analogy with other insects it is probable that this feature reflects the incorporation of lipoid in the waterproofing membrane.

Water droplets spread readily over the surface of the puparial shell so that the lipoid membrane appears not to be superficial. But since gentle abrasion of the puparium causes a three- to fourfold increase in the rate of transpiration the limiting barrier is clearly situated near the surface. It seems likely that the lipoid membrane which limits permeability to water is epicuticular, but protected by a layer of cement as in many other insects (Wigglesworth 1945).

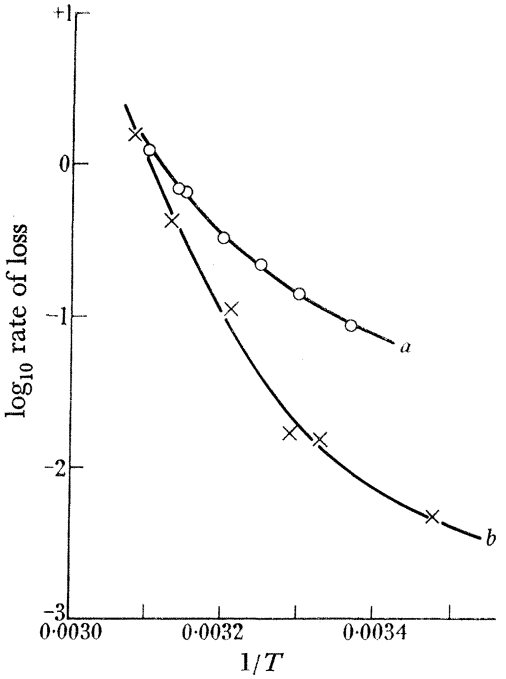

FIGURE 8. The transpiration of 3rd instar puparia (*a*) and of pupae (*b*) in dry air at different temperatures. The $\log_{10}$ rate is plotted as a function of the reciprocal of the absolute temperature.

(*b*) *The 4th instar larva*

The permeability of the 4th instar is slightly lower than that of the 3rd (cf. figures 2 and 3): this could be accounted for on the basis that a second membrane, of fairly high permeability—namely, the 4th instar cuticle—has been laid down inside the puparial shell. The vapour-pressure gradient between tissue fluids and atmosphere would then not exert its full force across the puparial shell. The transient increase in transpiration rate which occurs at the last larval moult (see figure 1, day 5) is in accord with this interpretation.

(*c*) *The pupa*

The permeability of the puparium after pupation is very much reduced; at this time blocking of the polypneustic lobes has no measurable effect on the rate of transpiration, presumably because the supernumerary stigmata are no longer in communication with the tracheal system, and diffusion through them is impeded by remnants of the 3rd instar air chambers. When puparia are maintained at 0% r.h. the amount of water which escapes through the pupal spiracle is minimal, so the rate of transpiration under these conditions can be taken as the nearest approximation to a measure of permeability. The value is 0·0005 mg $cm^{-2}\,h^{-1}(mm\,Hg)^{-1}$ which is very much lower than the permeability of most insect cuticles.

This reduction in permeability must be attributed to the deposition inside the puparial shell of the thin pupal skin; that lipoids are the effective waterproofing constituent of this skin is suggested by the marked temperature sensitivity of the transpiration (see figure 8, curve *b*).

Deposition of water droplets on the pupal skin shows that lipoid is laid down as the outermost layer of the cuticle, and there is no protective covering of cement. There seems to be some contamination of the inner surface of the puparial shell with this lipoid, since it can be shown to be strongly hydrophobic after completion of the pupal skin, whilst during the larval stages water spreads readily on the inner surface. The high rates of water loss on the penultimate day of pupal development indicate that the permeability of the puparial shell is not markedly affected by this contamination.

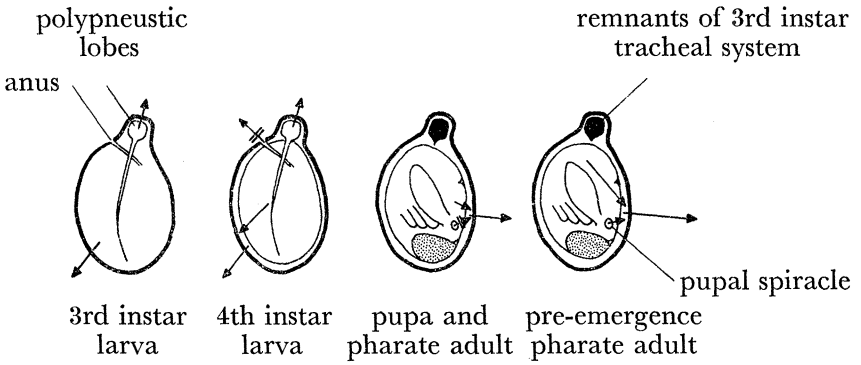

FIGURE 9. Diagrammatic summary of pupal water balance (for explanation see text).

(5) *Sex differences*

Willett (1953) showed that by the time of pupation, puparia of males have lost more weight than those of females; this difference has been confirmed in the present investigation. By analyzing the data for separate parts of the early period it has been shown that this sex difference is a simple consequence of differences in the speed of development. For there is no difference between males and females when the transpiration rates of the 3rd instar larva, the 4th instar larva or the pupa are compared; but the females pupate about 1 day sooner than the males, which means that the duration of the early and highly permeable period is shorter and hence the amount of water lost is smaller.

There is a difference between males and females on the last day of the pupal period, the rate of transpiration of females being lower than that of males. The reason for this difference has not been determined.

(6) *Summary*

By way of recapitulation the findings have been summarized in figure 9 which shows the processes operating at different stages of pupal development. Sites of water loss are indicated by arrows, the length of the arrow being proportional to the rate of loss; where active regulation occurs a double stroke has been drawn across the arrow.

(*a*) In the 3rd instar water is lost by transpiration through the supernumerary stigmata and through the puparial shell.

(*b*) In the 4th instar water is lost by transpiration through the supernumerary stigmata; by transpiration through the 4th instar integument to the air space between the larva and

the inner surface of the puparial shell, and from there through the puparium to the outside; and by excretion through the anus, the amount of water excreted depending on the amount previously lost.

(*c*) In the pupal and pharate adult stages the supernumerary stigmata are blocked by remnants of the 3rd instar tracheal apparatus and no water is lost through them. Water is lost into the air space between pupal skin and puparial shell partly by transpiration through the pupal skin and partly by way of the pupal spiracles; the latter component is under active control and the rate of loss depends on the water content of the pupa. From the air space water vapour diffuses out through the puparial shell, the combined rate of loss being about a third of that which characterizes the larval stages.

(*d*) A day or two before emergence spiracular regulation ceases and the permeability of the pupal skin increases considerably, resulting in a greatly increased rate of diffusion from the air space through the puparial shell.

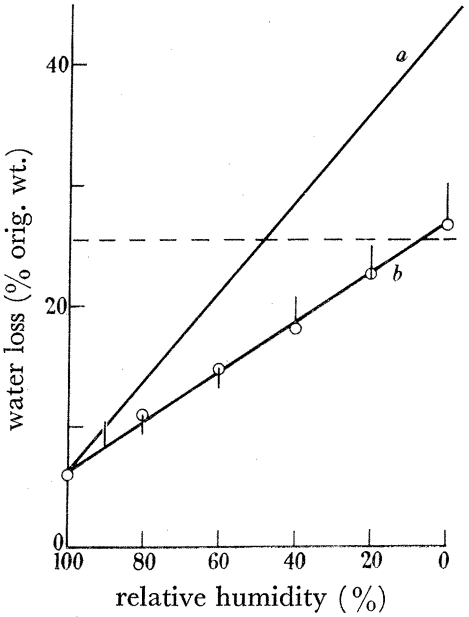

FIGURE 10. The percentage loss of weight during the pupal period as a function of humidity. (*a*) in the absence of excretory and spiracular regulation, (*b*) in normal pupae; ○, calculated values; and |, fiducial limits of observed values. For the purposes of calculation the pupal period was taken as 31 days, with the duration of the 3rd instar as 1 day, of the 4th instar 3 days and of the pupa 27 days of which the last 2 days are characterized by pre-emergence rates (for further explanation see text).

To get an idea of the importance of the regulatory powers one may calculate, from the data given in figures 2, 3 and 5, what would be the loss during the course of development in the absence of regulation. This has been done in figure 10 for comparison with a calculated curve for normal pupae, also based on figures 2, 3 and 5, and with observed values. The agreement between the observed and calculated curves (*b*) is very good, and it is interesting to note that a rectilinear relation between percentage loss and humidity results from the summation of two non-linear curves, one convex to the abscissa (curve *a*, figure 3) and one concave to the abscissa (curve *b*, figure 5). Figure 10, curve *a*, shows the water loss in the absence of regulation, that is, assuming that 6·1 % is lost by excretion at all humidities,

and that the transpiration of pupae is the same whether the early stages have been spent in 0 % r.h. or in 98 % r.h. (from figure 5, curve *a*). The line rises much more steeply from its origin, and the loss in dry air is more than 160 % of the regulated rate. By means to be discussed below (p. 203) it can be shown that a loss of 26 % of the original weight of pupae may be sustained without loss of viability; a line corresponding to this value has been included in figure 10, and it is seen that the critical point would, in the absence of regulatory control, be reached at a humidity of 52 % r.h., whereas normal pupae do not reach this threshold until humidity falls below 11 % r.h. In other words the powers of regulation possessed by pupae of *G. morsitans* enable them almost to double their range of tolerated humidity.

PART II. THE HUMIDITY OF PUPAL SITES

It has been shown in the preceding section that the rate of transpiration during the middle period of pupal development is a function of the humidity to which the earlier stages have been subjected. This finding opens the possibility of determining the humidity of the pupal site by using the pupa itself as a hygrometric instrument. Such a method has the advantage of direct measurements, in that the conditions under which humidity is measured are those actually experienced by the pupa; with methods involving soil samples or the insertion of instruments into the soil the possibility cannot be excluded that an alteration of soil structure may vitiate the results. No claim to accuracy can be made for the present method since considerable errors are involved in the estimate of pupal transpiration, but it has proved capable of giving an unequivocal answer to the question whether there occur seasonal fluctuations in soil humidity. In what follows the term 'soil humidity' will be used to denote the humidity which obtains in the air spaces of the soil.

Methods

The principle of the investigation was to compare the transpiration rates of pupae brought in from the field with those of pupae bred in the laboratory and maintained at known humidities during the early stages. Since laboratory-bred pupae are substantially smaller than wild pupae a systematic error will be introduced into the comparison unless it be made in terms which are independent of size. For this pupose weight is not a suitable function; the rate of loss during the mid-pupal period, expressed as a percentage of the original weight of the pupa, is negatively correlated with weight (mean z for five different humidities was $-0{\cdot}26$; $N=120$, $t=2{\cdot}74$, $P=0{\cdot}01$). In other words the larger pupae lose relatively less than the smaller ones. This could be expected if water loss were a function of surface area, since the larger pupae would have relatively small surface areas. And indeed, if loss is expressed in terms of surface area, no correlation can be demonstrated between size and rate of loss (mean $z=+0{\cdot}107$; $t=1{\cdot}08$, $P=0{\cdot}3$). In other words, when the rate of transpiration is expressed in terms of surface area it is independent of size and thus satisfies the criterion requisite for a comparison between wild and laboratory-bred pupae.

Surface area was estimated on the assumption that the pupa is a sphere of diameter equal to half the sum of its length and breadth. The error involved in this assumption was estimated by determining the area of a celloidon skin deposited on pupae and subsequently

stripped off and projected on squared paper. The calculated figure was about 15 % greater than the more direct estimate; but the percentage excess was the same for very large (*G. brevipalpis*) and very small (*G. austeni*) pupae, so there was little risk of significant systematic errors. The formula was adopted on the grounds of greater convenience as compared with one which would describe the shape of the pupa more accurately.

Marked seasonal fluctuations in temperature are known to occur in pupal sites (Nash 1939; Harley 1954) and it is necessary to consider whether these will affect the amount of water lost during early pupal stages and so influence mid-pupal transpiration rates. Some experiments were carried out at 19·7° C and at 30·8° C for comparison with results already obtained at 24·7° C. The data are set out in table 4. It is clear that in spite of the marked differences in saturation deficiency, the loss of water is the same at the three different temperatures; this is in accord with the findings of Jack (1939, table XLI). The reason for this apparent anomaly is that as the saturation deficiency is increased at the higher temperatures, so is the rate of development speeded up; the rate of transpiration is high in accord with the high saturation deficiency, but the early and highly permeable stages are passed through more rapidly. Over the range in question the two opposed tendencies balance each other so perfectly that the total amount of water lost is uninfluenced by temperature. This means that for the purposes of studying the humidity of pupal sites by the present method, seasonal fluctuations in temperature may be ignored.

TABLE 4. THE EFFECT OF TEMPERATURE ON THE LOSS OF WATER FROM PUPAE OF *GLOSSINA MORSITANS* DURING THE FIRST 8 DAYS OF PUPAL LIFE

relative humidity = 40 %.

temperature (° C)	saturation deficiency (mm Hg)	loss of water (%)	S.E.	*N*
19·7	10·3	10·1	±0·83	6
24·7	14·1	9·7	±0·26	13
30·8	20·0	9·9	±0·64	14

S.E. = standard error.
N = number of pupae.

Pupae, collected at Singida in the Central Province of Tanganyika, were brought in from the field and exposed for 24 h to 0 % r.h. before transpiration rates were determined. Weight loss during the following 6 days was then recorded and the rate of loss expressed as a function of surface area. From the emergence date of the flies the age of pupae at the time of desiccation could be determined, and to make the results comparable with those for laboratory-bred pupae (see p. 185) only individuals which were more than 9 days old at the beginning of the experiment, and less than 19 days old at the end, were included in the estimate of mean transpiration rate.

As a check on these calculations the amounts of water lost by the pupae during their early stages in the field were estimated. From a curve relating the weight of pupae newly deposited in the laboratory to their surface area, it was possible to gauge the deposition weight of wild pupae knowing their surface area. The difference between this original weight and the weight of the pupae on arrival at the laboratory is a measure of the amount of water lost during the early stages.

Results

The results of a series of experiments covering two dry and one wet season are shown in figure 11 and table 5. The transpiration rates of wild pupae are given in figure 11*b* with relative humidities corresponding to given rates of transpiration shown on the right-hand ordinate (derived from figure 6, p. 186); the rainfall is shown in figure 11*c*. It is clear that

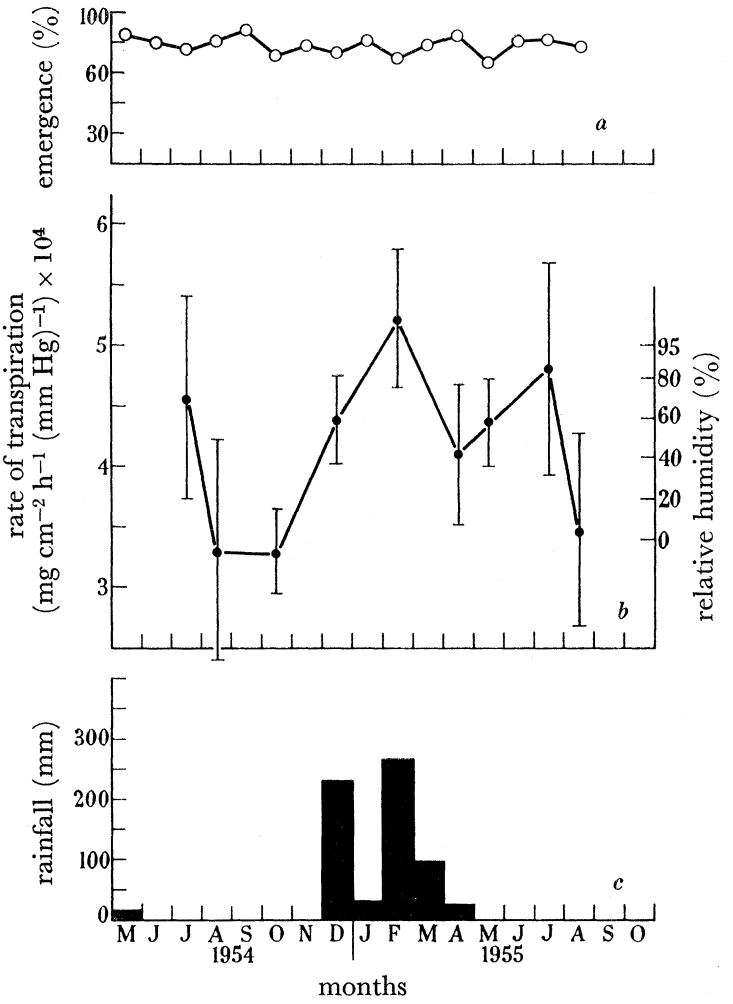

FIGURE 11. Seasonal variations in mortality (*a*) and in the rate of transpiration (*b*) of *G. morsitans* pupae in relation to the monthly rainfall (*c*).

there are marked seasonal fluctuations in transpiration rate with low values at the end of the dry season (August to October) and high values during the rains, with the early dry season intermediate. These fluctuations are reflected in column 10 of table 5, which shows that the difference between the original weight of the pupae and their weight on arrival is greatest in October, least in December and February with intermediate values for the other months of the year.

The marked rise in transpiration rate which occurs between October and December to February can reasonably be attributed to an increase in soil humidity following the onset of the annual rains; the lower values in April, May, June and July to their cessation. But a major fall occurs between July and August some 2 to 3 months after the last rain was recorded.

This second drop in soil humidity, which cannot be correlated with any climatic change, is probably caused by annual fires, which in 1955 swept the collecting area on 19 July. Stands of grasses and leafy shrubs surrounding the pupal sites would be likely to maintain a fairly high humidity, in part by transpiration from their green parts and in part by reduction of air movement. After burning there would be a fall in the humidity above the pupal sites and this would be reflected in a drop of the soil humidity.

TABLE 5. SEASONAL VARIATIONS IN PUPAL TRANSPIRATION RATE AND EARLY WEIGHT LOSS

1 year	2 month	3 transpiration rate (mg cm^{-2} h^{-1} (mm Hg)$^{-1}$) $\times 10^4$	4 s.e.	5 N	6 variance $\log_e$	7 surface area (cm^2)	8 original weight (mg)	9 arrival weight (mg)	10 difference (mg)
				Glossina morsitans					
1954	July	4·56	±0·42	8	−1·748	0·516	29·8	27·5	2·3
	August	3·30	±0·46	5	−1·537	0·512	29·5	27·1	2·4
	October	3·28	±0·19	9	−3·224	0·522	30·3	25·6	4·7
	December	4·38	±0·18	8	−3·381	0·486	27·3	25·7	1·6
1955	February	5·21	±0·29	7	−2·501	0·534	31·3	30·9	0·4
	April	4·10	±0·29	5	−2·465	0·558	33·3	31·1	2·2
	May	4·36	±0·18	7	−3·411	0·558	33·3	30·9	2·4
	July	4·81	±0·44	8	−1·640	0·542	32·0	30·1	1·9
	August	3·46	±0·40	5	−1·826	0·537	31·6	29·2	2·4
				G. swynnertoni rot hole sites					
1954	October	3·28	±0·18	7	—	0·473	26·7	23·5	3·2
1955	July	4·49	±0·17	13	—	0·510	29·4	27·3	2·1
1956	July	3·99	±0·17	11	—	0·532	31·2	27·1	4·1
				G. swynnertoni log sites					
1956	July	4·06	±0·16	6	—	0·490	27·6	25·1	2·5
				G. pallidipes					
1954	October	4·33	±0·27	6	—	0·640	40·2	34·8	5·4

s.e. = standard error. N = number of pupae.
For comparison of variability, given n_1 not less than 4 and n_2 not less than 6, any difference in $\log_e$ variance greater than 1·5 is significant at the 5 % level of probability.

The variability of transpiration rate within a sample of pupae also shows marked seasonal fluctuation (see table 5, column 6). The variance during July and August is, in both years, significantly higher than in October, December and May. It may be that this difference reflects a difference in the heterogeneity of pupal sites; in other words towards the end of the dry season all pupal sites have low soil humidities, and during the rains all pupal sites have high soil humidities, while during the transition period in August and July some pupal sites have low and some high soil humidity so that pupae sampled at this season show greater variability.

Figure 11*b* shows that during the dry season the humidity of pupal sites in a habitat of *G. morsitans* may reach very low values during part of the year; but that this drying out of the pupal sites is without effect on the viability of pupae is shown in figure 11*a*, where the percentage mortality is plotted for the period in question. The values are seen to fluctuate around a mean of about 80 % without any evidence of regular seasonal variation. It seems that the pupa is so well buffered against changes of relative humidity, by mechan-

isms described in the previous section, that it is unaffected by such seasonal changes as may occur in their natural environment. This confirms the conclusion reached by Jack (1939) in the course of a study of *G. morsitans* in Southern Rhodesia.

A few experiments have been done to determine the humidity of pupal sites in the habitat of *G. pallidipes* and *G. swynnertoni* at Shinyanga (see table 5). Pupae of these species can be obtained in sufficient numbers only during the dry season. The values obtained for *G. swynnertoni*, when compared with values obtained in the laboratory as for *G. morsitans*, reflect a decrease in the humidity of pupal sites from about 80 % r.h. in April (beginning of the dry season) to about 20 % at the height of the dry season. It has not been possible to demonstrate any difference between pupae obtained from log sites and from rot holes, which suggests that the pronounced shift to rot hole sites which occurs with the onset of the hot dry season (Burtt 1952) is related to some factor other than soil humidity.

The *G. pallidipes* pupae were obtained from deciduous riverine thicket; they give evidence of a soil humidity of about 40 % r.h. during October, which is somewhat higher than those simultaneously obtaining in the *G. swynnertoni* sites.

Discussion

The results of the present investigation are at variance with the conclusions reached by Buxton (1936) who studied the properties of soils taken from pupal sites of *G. submorsitans* in Northern Nigeria. This author maintained that even when soil appears to be quite dry the atmosphere of the soil spaces may be very nearly saturated. He suggested that the reason why pupal mortality remained low even at the end of the dry season was that the humidity of pupal sites never fell far below saturation; but the experimental basis of these conclusions is open to question.

The method consisted essentially in determining the moisture content of soil samples in equilibrium with various relative humidities. It was found that soil in equilibrium with 90 % r.h. contained as little as 4 % of water and appeared quite dry; the converse inference was made, that soil which is in appearance quite dry may be in equilibrium with 90 % r.h. which will be the humidity of the soil spaces. But this inference does not apply to the soil of pupal sites which is not in a closed system. Since the humidity of the ambient atmosphere may in the dry season reach very low values (mean diurnal humidities as low as 20 % r.h. have been recorded at Shinyanga) is is clear that a gradient must exist between the dry atmosphere and the damp (it is believed nearly saturated) air found in the interstices of the soil at a depth of 20 cm or so. The present investigation has shown that this gradient is such that pupae buried to a depth of 1 to 6 cm may be exposed to humidities which approximate closely to those of the atmosphere.

That a decrease in soil humidity occurs during the dry season is confirmed by some data published by Jack (1939, table IV). Using *G. morsitans* from Southern Rhodesia he determined the water content of flies immediately after their emergence from wild pupae, and recorded a decrease from about 76·5 % during the rainy season to about 75·0 % towards the end of the dry season. This difference was so slight as to be ignored by the author; but comparison with his table XLI indicates that the difference represents a shift in soil humidity from about 65 % to about 30 % r.h. Relative humidities measured at 8.30 a.m. during the corresponding period fell from 87 to 45 %.

It may be concluded that in some, and probably in most, of the habitats of such species as *G. morsitans*, *G. swynnertoni* and *G. pallidipes* the humidity of the pupal sites decreases during the dry season; and that in areas where the dry season is prolonged and where pupal sites are exposed to grass fires the humidity may reach very low values.

PART III. COMPARATIVE ASPECTS OF PUPAL WATER BALANCE

The results presented in the preceding sections have shown that the humidity of pupal sites may reach low values during the last months of the dry season, but that pupae of *G. morsitans* are sufficiently well waterproofed to withstand such fluctuations in soil humidity. The question arises whether the same is true of all species, and if not, whether species which are less resistant to desiccation are limited to areas in which soil humidity may be expected to be near saturation at all times of the year.

Material

The species which were available for study are listed below, together with the localities from which they were obtained:

		lat.	long.
G. brevipalpis Newstead	Mkata, Tanganyika	7·0 S.	37·5 E.
G. fuscipleuris Austen	Masindi, Uganda	2·5 S.	31·0 E.
G. longipennis Corti	Wembere, Tanganyika	4·0 S.	34·5 E.
G. palpalis fuscipes Newstead	Kuja River, Kenya	1·0 S.	34·5 E.
G. pallidipes Austen	Shinyanga, Tanganyika	3·5 S.	33·0 E.
G. morsitans Westwood	Singida, Tanganyika	5·0 S.	35·0 E.
G. swynnertoni Austen	Shinyanga, Tanganyika	3·5 S.	33·0 E.
G. austeni Newstead	Kilifi, Kenya	3·5 S.	40·0 E.

Results

(1) *The resistance to desiccation of different species*

Figure 12 shows the mortality of tsetse pupae as a function of relative humidity. With many of the species only small samples were available, and in these circumstances chance mortality may reduce the percentage emergence considerably. The range of emergences recorded in 90 to 98 % r.h., that is when death by desiccation can be excluded, is about 25 %, so that a percentage emergence as low as 75 % may occur irrespective of humidity. Depression of the emergence rate below this value is taken as indicating a deleterious effect of humidity.

The conditions which can be tolerated are seen to vary widely; with *G. brevipalpis* at one extreme—non-viable at humidities below 60 %, and *G. longipennis* at the other—completely viable at 0 % r.h. The situation bears no relation to the phylogenetic grouping of the species (see below, p. 204), since resistant and susceptible members occur both in the primitive *Fusca* group (*G. longipennis* and *G. brevipalpis*) and in the specialized *Morsitans* group (*G. swynnertoni* and *G. austeni*). This suggests that convergent evolution may have played a part, but before the question can be discussed further it will be necessary to consider in some detail the factors responsible for these differences in resistance to desiccation.

It has been shown that the water balance of *G. morsitans* pupae is based on two active mechanisms, one of excretion and one of spiracular regulation, operating on a background of low membrane permeability, of puparium and pupal skin, respectively. Any of these

factors might be involved in the development of resistance to desiccation. Another possible basis for specific differences is the amount of water which may be lost before the water content reaches a level preventing further development. This in turn will depend on two factors, namely, the range of percentage water content which can be tolerated and the size of pupae; for the same absolute water loss will represent a higher percentage in a species with a small pupa than in one in which the pupa is larger. The available data on all

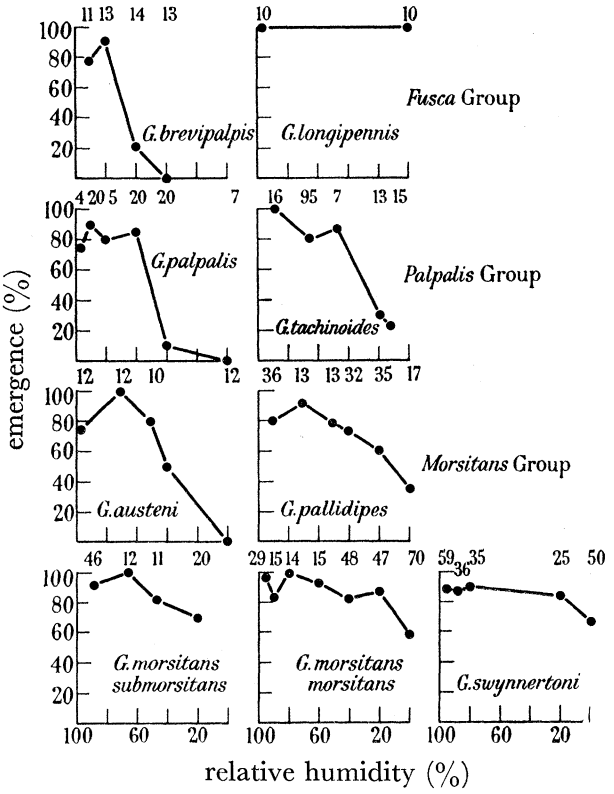

FIGURE 12

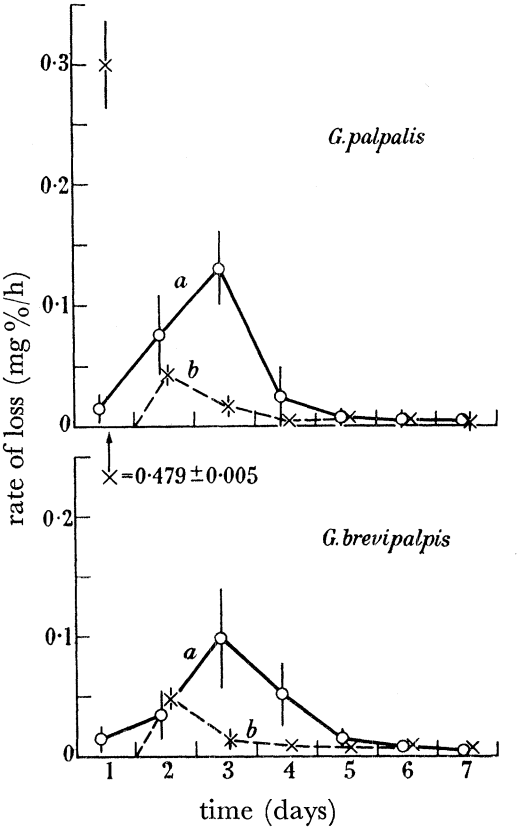

FIGURE 13

FIGURE 12. The percentage emergence of different species as a function of the relative humidity obtaining during the whole of the developmental period. Data for *G. morsitans submorsitans* and *G. tachinoides* from Buxton & Lewis (1934); all values obtained at 24° C except for *G. tachinoides* at lower humidities where data are available for 30° C only, so the percentage emergence may be somewhat underestimated relative to the other species. Pupae deposited in the laboratory are on an average about 10 % smaller than pupae collected in the field. Analysis for the effect of size on emergence at suboptimal humidities for a number of different species shows that a size difference of this order would decrease the emergence by about 20 %; this is equivalent to an increase in humidity of about 10 %, as judged from the slopes of the mortality curves. Thus, all the curves of this figure should be displaced to the right by an amount corresponding to 10 % r.h.; and the same applies to the ranges shown in figure 14 which should be extended by 10 % r.h. towards the dry. To avoid confusion statements in the text will refer to laboratory-bred pupae, but they must be accepted with the above qualification. The figures at the top of each graph shows the number of pupae tested at each humidity.

FIGURE 13. The rate of water loss at 98 % r.h. during the 4th larval instar of *G. palpalis* and *G. brevipalpis*. (*a*) puparia maintained at 98 % r.h. throughout. (*b*) puparia maintained at 0 % r.h. on the first day (as for figure 4).

these points have been assembled in tables 6 and 7 and in figure 13, and the different factors will be discussed in turn.

(2) *The basis of specific differences in resistance to desiccation*

(*a*) *Active mechanisms*

(i) *Excretory regulation.* The phenomenon of excretory regulation has been demonstrated for all the three groups of tsetse fly, the *Fusca* group, the *Morsitans* group and the *Palpalis* group. Figure 13 shows the loss of weight in saturated air for *G. palpalis* and *G. brevipalpis* (cf. figure 4 for *G. morsitans*); it is clear that in pupae whose water contents have been depleted by previous exposure to dry air the rate of excretion is reduced to a fraction of its former value. This reduction is even more marked in *G. palpalis* and *G. brevipalpis* than in *G. morsitans*, related presumably to the much greater depletion which occurs during the first day in the former species. It is clear that it is not excretory regulation which is responsible for the superior resistance of *G. morsitans* to desiccation.

TABLE 6. THE WATERPROOFING OF PUPARIUM AND PUPAL SKIN OF DIFFERENT SPECIES

1 group	2 species	3 surface area (cm^2)	4 puparium loss mg cm^{-2} h^{-1} (mm Hg)$^{-1}$ $\times 10^3$	5 loss through puparial shell mg cm^{-2} h^{-1} (mm Hg)$^{-1}$ $\times 10^3$	6 lobe loss (mg %/h $\times 10^2$)	7 pupal loss mg cm^{-2} h^{-1} (mm Hg)$^{-1}$ $\times 10^4$ maximum	8 pupal loss minimum	9 difference
Fusca	*brevipalpis*	0·95	16·7 ± 0·74	10·2 ± 0·39	18·2 ± 1·5	9·4 ± 0·42	7·9 ± 0·93	1·5
	fuscipleuris	0·95	14·3	—	—	9·7	7·7	2·0
	longipennis	1·03	5·4 ± 0·36	—	—	6·5 ± 0·31	3·9 ± 0·14	2·6
Palpalis	*palpalis*	0·55	7·1 ± 0·43	3·5 ± 0·22	15·9 ± 2·9	7·2 ± 0·18	4·2 ± 0·31	3·0
	tachinoides	0·37	3·4	—	—	—	3·3	—
Morsitans	*pallidipes*	0·68	3·4 ± 0·14	2·9 ± 0·17	2·9 ± 1·0	5·6 ± 0·12	3·0 ± 0·02	2·6
	morsitans	0·53	2·9 ± 0·26	1·7 ± 0·07	7·6 ± 0·8	5·5 ± 0·18	3·1 ± 0·09	2·4
	submorsitans	0·52	4·7	—	—	—	3·0	—
	swynnertoni	0·51	2·5 ± 0·26	1·6 ± 0·17	2·7 ± 2·1	5·1 ± 0·19	2·8 ± 0·19	2·3
	austeni	0·39	6·3 ± 0·20	3·2 ± 0·18	17·7 ± 2·6	5·4 ± 0·31	3·0 ± 0·18	2·5

Surface areas were calculated from measurements of pupae collected in the field.
Data for *G. tachinoides* and *G. morsitans submorsitans* were taken from the graphs of Buxton & Lewis (1934) and values calculated relative to *G. morsitans* exposed to identical conditions.
Pupae of *G. fuscipleuris* were not available in sufficient numbers to warrant an estimate of variability.

(ii) *Spiracular regulation.* It is possible to get some idea of the power of spiracular regulation by comparing rates of water loss of pupae maintained in saturated air, regulation then being minimal, with pupae whose water content has been depleted by exposure to dry air and in which, consequently, water loss through the spiracles will be stringently controlled. This comparison is set out in the last three columns of table 6, the power of regulation being reflected in the magnitude of the difference (last column). It is clear that there is no marked difference between species in this respect. *G. brevipalpis* constitutes an apparent exception; but with this species the number of pupae available was small, the errors correspondingly large and the difference between this and other species of dubious significance.

It is reasonable to conclude that neither of the active mechanisms of regulation are implicated in the observed differences in resistance to desiccation.

(*b*) *Waterproofing membranes*

(i) *The puparium.* Table 6, column 4, shows the rate of loss in dry air of the 3rd instar puparium. Except for *G. longipennis* the waterproofing of the puparium is seen to be closely

related to resistance to desiccation; with *G. brevipalpis* as the most susceptible species having the least waterproof puparium; followed in order by *G. palpalis*, *G. austeni*, *G. pallidipes* and *G. morsitans*, with *G. swynnertoni* as the last member of the series, having the most impermeable puparium and the highest resistance to desiccation. *G. longipennis* is viable in dry air despite its relatively high permeability, but this apparent discrepancy is a function of its large size as will be discussed below.

The figures in column 4 refer to the over-all loss from the puparium; unfortunately, data which enable this loss to be partitioned between lobe loss on the one hand, and transpiration through the puparial shell on the other (cf. p. 191), are only available for some of the species. As far as they go they correspond fairly closely to the figures for over-all loss (see column 5), indicating that an increase in the efficiency of the actual waterproofing barrier has been an important factor in conferring resistance to desiccation.

It has been shown that a considerable amount of water escapes through the polypneustic lobes of the 3rd instar puparium, and it seemed possible that the relative size of lobes might also be significant in relation to the waterproofing of the puparium as a whole. Only members of the *Palpalis* and *Morsitans* groups have been considered in this connexion, the form of lobes in the *Fusca* group being radically different. Figure 16, plate 10, shows puparia of the species for which data are available. *G. austeni* and *G. palpalis* are readily distinguished from the others by their relatively massive lobes; with the delicately lobed *G. swynnertoni* at the opposite extreme. The values for lobe loss have been included in table 6 (column 6), and they are seen to conform fairly closely to differences in relative lobe size, with the percentage loss for *G. austeni* and *G. palpalis* 2 to 3 times greater than values for the smaller lobed species. Reduction in lobe size may thus have been another factor of importance in the evolution of resistance to desiccation.

(ii) *The pupa.* It has been shown that with pupae maintained in dry air from the time of puparium formation, water loss through the spiracles is reduced, so that under these conditions the rate of transpiration furnishes the nearest approach to an estimate of the permeability of the pupal skin. The relevant figures are set out in column 8 of table 6. On the basis of this character the members of the *Morsitans* group form a natural unit set apart from the rest of the species studied by their low permeabilities. Within each of the other groups there has been a reduction in the permeability of the pupal skin associated with an increase in resistance to desiccation; and taking the *Morsitans* group as on the whole the most resistant of the three, the findings suggest that a decrease in permeability of the pupal skin has also played a part in the evolution of efficiently waterproofed pupae.

(*c*) *The quantity of water reserves*

(i) *The tolerated range of water content.* An estimate of the extent to which water content may be depleted without loss of viability was obtained by exposing pupae to two relative humidities, one of which was known to give more than 50 % mortality the other less than 50 %. The amount of water lost by the survivors up till the last day of pupal life was measured, and by interpolation the percentage loss under conditions which would give 50 % mortality was calculated. The results are set out in table 7, and the species are seen to fall into two main groups; those which can tolerate a loss of about 29 % of their original weight, representing 40 % of the amount of water originally present in the pupa

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(*G. longipennis*, *G. morsitans* and *G. swynnertoni*); and those which cannot complete their development when losses exceed about 24 % of their original weight (*G. brevipalpis*, *G. palpalis* and *G. austeni*). With regard to *G. swynnertoni* the difference seems to depend on a real change in what may be called the 'critical water content'; for reference to column 4 shows that the water content of its pupae at the time of deposition does not differ significantly from values obtained with *G. palpalis* and *G. pallidipes*. The initial water content of *G. morsitans* pupae, on the other hand, is significantly higher than that of the other species studied, suggesting that in this species a greater quantity of expendable reserves may be laid down at the beginning of development. But the difference is insufficient to account for the observed difference in tolerated loss (being equivalent to only 2·5 % of the original weight) so it seems that there may in addition have been a shift in the critical water content.

TABLE 7. THE PERCENTAGE WATER LOSS TOLERATED BY PUPAE OF DIFFERENT SPECIES, AND THE EXPENDABLE RESERVES

species	weight (mg)	loss during the pupal period (% of original weight)	initial water content (%)	expendable reserves (mg)
G. longipennis	80	30·0 ± 1·02	—	24·0
G. brevipalpis	78	24·8 ± 1·31	—	18·7
G. palpalis	32	24·2 ± 0·42	74·27 ± 0·32	7·7
G. pallidipes	43	24·5 ± 0·78	73·76 ± 0·24	10·5
G. morsitans	31	28·4 ± 0·93	75·02 ± 0·32	8·8
G. swynnertoni	29	29·2 ± 1·27	73·91 ± 0·26	8·5
G. austeni	19	24·5 ± 1·12	—	5·3

The low tolerance group includes the species susceptible to desiccation, the high tolerance group the resistant species, which suggests that the provision of extra reserves of expendable water has been an important factor in the evolution of resistance to desiccation.

(ii) *The size of pupae*. The weights of pupae of different species are included in table 7. The importance of these differences in size may be illustrated by considering two hypothetical species, one the size of *G. longipennis* the other the size of *G. austeni*; both with a puparium permeability of $3{\cdot}4 \times 10^{-3}$ and a pupal skin permeability of 5×10^{-4} mg cm^{-2} h^{-1} (mm Hg)$^{-1}$. Taking the pupal period as 30 days the loss in dry air of the small species would amount to 6·37 mg or 42·5 % of its original weight; while for the large species the loss would be 17·78 or 23·7 % of the original weight. Taking 28 % as the critical level of water loss, the difference in size between *G. longipennis* and *G. austeni* would make the difference between complete viability and 100 % mortality.

Knowing the level to which water content may be reduced and the size of the pupa, it is possible to calculate the amount of expendable reserve for the different species. This is shown in the last column of table 7; the reserves of *G. longipennis* are almost five times as great as those of *G. austeni* with the other species taking intermediate values.

(3) *Resistance to desiccation in relation to habitat*

Very little is known about the phylogeny of tsetse flies, but on the basis of the structure of male genitalia it is possible to divide the genus into groups of increasing complexity (Newstead, Evans & Potts 1924):

(i) The primitive *Fusca* group comprising the ten largest species of tsetse fly.

(ii) The *Palpalis* group with male armatures bearing a strong resemblance to those characteristic of the *Fusca* group.

(iii) The specialized *Morsitans* group, with male armatures of very distinctive form, but sharing one important feature with members of the *Palpalis* group, namely, the connecting membrane which extends between right and left superior claspers.

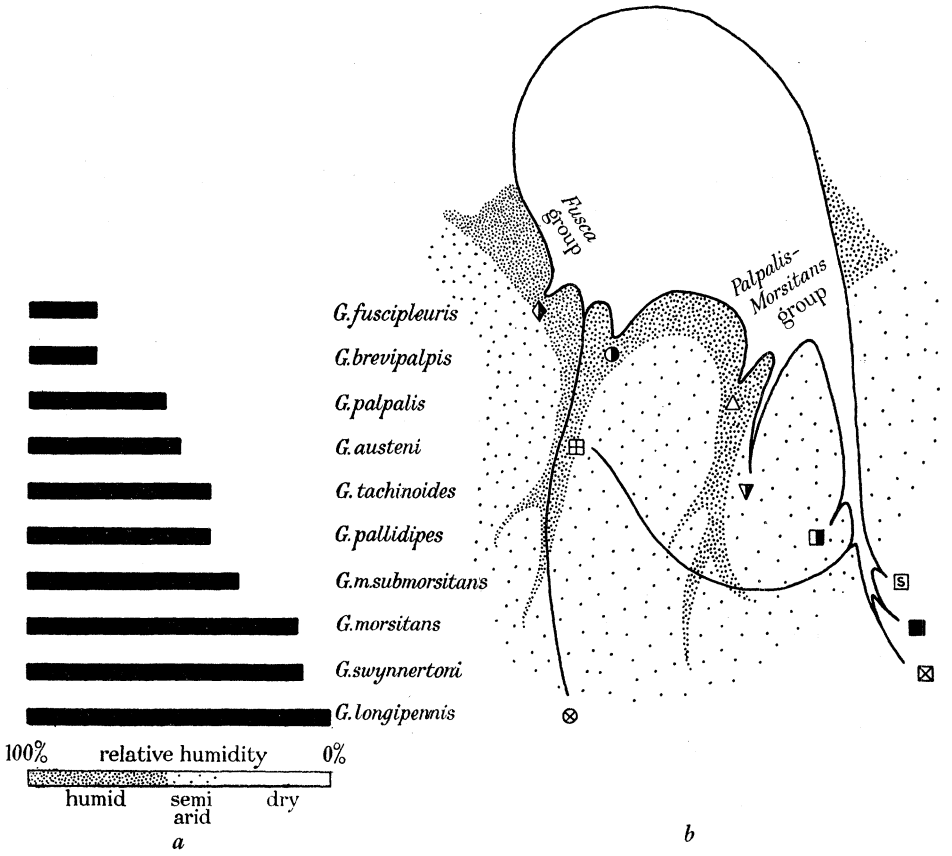

FIGURE 14. Diagrammatic representation of the relation between habitat and resistance to desiccation. (*a*) the ranges of relative humidity which can be tolerated without loss of viability (see note to figure 12). (*b*) a possible phylogeny of some species of tsetse fly, based on pupal water balance. The number of *Glossina fuscipleuris* pupae available was not sufficient to give an estimate of its tolerated range; it has been placed with *G. brevipalpis* which it closely resembles in respect of water loss (see table 6).

All but one member of the *Fusca* group are associated with tropical rain forest, or rain forest relicts, and it is reasonable to imagine that this type of vegetation represents the ancestral habitat. Granted this assumption, the habitats of other species may then be defined by their degree of divergence from the ancestral type. On this basis it is convenient to recognize three main divisions.

(*a*) *Hygrophytic habitats*

Habitats closely resembling the ancestral and characterized by evergreen forest. Such vegetation is limited to regions of high rainfall evenly distributed over the year; or, in places where a dry season occurs, it is closely associated with permanent free water.

Of the species studied during the present investigation the habitats of *G. brevipalpis* and *G. fuscipleuris* may be said to conform most closely to the ancestral type. Apart from an

isolated pocket east of Lake Victoria *G. fuscipleuris* is limited to the western parts of Uganda, where it overlaps with the true rain forest species like *G. fusca*; *G. brevipalpis* extends eastwards as far as the India Ocean, but in regions where there is a pronounced dry season it is invariably associated with the evergreen vegetation of major drainage lines. The distribution of *G. austeni* overlaps to a large extent that of *G. brevipalpis*, both geographically and topographically; and *G. palpalis* must also be included in this category, its distribution in East Africa being limited to the shores of the larger lakes and the rivers draining into them.

(*b*) *Semi-arid habitats*

Habitats in regions where a dry season may occur; but where the breeding places would be protected from the action of grass fires. Vegetation semi-deciduous, often associated with minor drainage lines.

In this class would be included *G. tachinoides*, whose breeding during the dry season is limited to 'residual forest islands' with peripheral thickets acting as buffers against the grass fires (Nash 1939); and *G. pallidipes*, typically a thicket species, whose breeding in the drier parts of its range is confined to riverine vegetation (Dr E. Burtt, personal communication).

(*c*) *Xerophytic habitats*

These occur in regions having a prolonged dry season, where the breeding sites are exposed to the action of grass fires.

In this category belong *G. morsitans submorsitans* which in the hot dry season of the northern parts of its range is to some extent dependent on riverine thickets, but elsewhere breeds in the savannah throughout the year; *G. morsitans* which is independent of thicket throughout its range; *G. swynnertoni* breeding in the arid thorn scrub of central Tanganyika; and *G. longipennis* which, although usually associated with drainage line vegetation (Lewis 1942), is not limited to thicket for purposes of breeding. In an area near Lake Kitangiri co-inhabited by *G. pallidipes*, *G. swynnertoni* and *G. longipennis* the two latter species were breeding in rock sites on the exposed slopes of a hill side during the last part of the dry season; the breeding of *G. pallidipes* was confined to riverine thicket in which pupae of *G. longipennis* were also found, but no *G. swynnertoni*.

With regard to the humidity of pupal sites in these three types of habitat, we have seen that in areas where a prolonged dry season occurs, and where the pupal sites are exposed to the action of grass fires, the humidity may drop to about 20 % r.h. during the height of the dry season. Before the onset of fires the soil humidity did not fall below about 40 % r.h. even after 3 to 4 completely rainless months (see figure 11); that this represents the lower limit for sites protected from the action of fires is confirmed by data for *G. pallidipes* obtained from riverine thickets in Shinyanga (see p. 199). In areas where rain falls every month, or where there is free water, and where pupae are deposited below evergreen vegetation it is unlikely that the soil humidity will fall far below saturation. Observations on the seasonal colour changes of *G. pallidipes* (which are humidity induced, see Burtt 1953) suggest that along the northern shores of Lake Victoria, in the area described by Chorley (1944) the humidity of pupal sites is between 80 and 90 % r.h. throughout the year (Glasgow & Bursell, unpublished observation).

The relations between habitat and the resistance to desiccation of pupae in terms of these three categories have been summarized in figure 14. Figure 14*a* shows the range of humidities which can be tolerated by different tsetse pupae without loss of viability (data from figure 12). It is clear that there is a very close correspondence between the type of habitat in which breeding takes place and the resistance of a species to desiccation. The three most susceptible species are found only in hygrophytic habitats, while the four most resistant species occur in the xerophytic habitats. Reference to tables 6 and 7 suggests that, broadly speaking, invasion of the semi-arid zone has been contingent on the development of more impermeable pupal integuments; while conquest of the xerophytic environment has involved in addition an increase in the quantity of expendable water reserves.

By way of summary the situation has been represented as a tentative phylogeny in figure 14*b*. This shows members of the primitive *Fusca* group confined within the limits of the ancestral habitat, all except *G. longipennis*. This species, which is by some considered the most xerophilous of all tsetse flies, owes its success in an arid environment in large part to its extensive reserves of water (see table 7). The permeabilities of its pupal membranes, although low compared with other members of the *Fusca* group, are still too high to have ensured drought resistance in any but a large tsetse fly.

In the *Palpalis* group the type member is limited by relatively poor waterproofing and inadequate reserves of water to the ancestral type of habitat, as manifested in East Africa along lake shores and river banks. In *G. tachinoides* the permeability of puparium and pupal skin is substantially lower, and this species has achieved partial independence of the forest habitat in spite of its small size.

In the *Morsitans* group *G. pallidipes* has permeabilities of the same order as *G. tachinoides*, but being a larger fly with greater reserves it shows greater resistance to desiccation. Its mortality increases at humidities below 40 % r.h. so in the drier parts of its range its breeding is limited to riverine thicket where pupal sites will be protected from grass fires.

G. morsitans and *G. swynnertoni* are set apart from the other members of the *Morsitans* and *Palpalis* group by being able to complete their development after a loss representing more than 28 % of their original weight. This means that in spite of their small size they have substantial reserves of water, and these, coupled with particularly low membrane permeabilities, have rendered them independent of any fluctuation in humidity which could occur in their habitat (at Shinyanga, for example, the lowest mean diurnal humidity recorded is about 20 % r.h.).

G. austeni occupies a rather anomalous position as far as pupal water balance is concerned, for its resistance to desiccation appears to be considerably in excess of that which would be necessary in the type of habitat in which it occurs. It is usually found associated with *G. brevipalpis*, but there is some indication that the vegetational requirements of the two species are not identical, *G. brevipalpis* preferring thickets of medium density and *G. austeni* tall and heavy thickets (Moggridge 1950). If anything then, the pupal sites of *G. austeni* should be more humid than those of *G. brevipalpis*, and resistance to humidities down to 80 % r.h. should suffice for *G. austeni* as it does for *G. brevipalpis*; yet *G. austeni* is completely viable at humidities less than 50 % r.h. This resistance it owes in large part to the low permeability of its pupal skin, which is on a level with that of the most resistant members of the *Morsitans* group. It seems possible that this low permeability, greatly in excess

of present requirements, might be a legacy from a time when the species inhabited semi-arid or arid environments like the other members of the *Morsitans* group. On this view its occurrence in evergreen thickets and forests would represent a secondary return of the species to the ancestral type of habitat, associated perhaps with a reduction in size which rendered even that degree of waterproofing inadequate to survival under arid conditions.

One may imagine that having reoccupied a hygrophytic environment, selection pressure for low permeability and small lobes would be relaxed, the efficiency of waterproofing of the puparium decreases (see table 6, column 5) and the relatively enormous lobes which characterize this species make their appearance. In this connexion it is significant that the coefficient of variation in lobe size is greater in *G. austeni* than in other members of the *Morsitans* group (12·4 compared with 9·3, 8·4 and 8·1 for *G. pallidipes*, *G. morsitans* and *G. swynnertoni*, respectively); secondly it is only in *G. austeni* that local races can be distinguished on the basis of lobe size. The size of lobes in a sample of *G. austeni* from the Kenya Coast was $4{\cdot}20 \pm 0{\cdot}21$ (arbitrary units), while for a sample from Lorenço Marques the value was $3{\cdot}72 \pm 0{\cdot}18$ (the size of the pupae was not significantly different being 0·391 and 0·389 cm^2, respectively). An analogous situation has been found in the *Palpalis* group where the matter, however, is complicated by differences in the size of pupae. Since there is no evidence of heterogony as regards the size of lobes in pupae of different size within a species, this difficulty may be overcome by taking the ratio between lobe size and surface area of the puparium. The values for *G. palpalis fuscipes* (from Uganda) is 0·97 compared with 0·77 for *G. palpalis martinii* (from Lake Tanganyika) and *G. palpalis palpalis* (from Kaduna in Northern Nigeria). It is tempting to relate these differences to the comparatively arid conditions obtaining in Kaduna, near the northern limits of *G. palpalis*, and along the eastern shores of Lake Tanganyika.*

The above discussion suggests that on the basis of pupal water balance it is not unreasonable to envisage the evolution of tsetse flies in terms of an invasion of progressively more arid environments, concomitant with the regression of tropical rain forest in Central Africa. A retreat of the ancestral habitat, whether occasioned by long-term climatic changes, or brought about by the activities of man, or both, would leave outrunners of gallery forest along the major drainage lines, thus enormously extending the front between primitive and prospective habitats, and increasing the opportunity for invasion. The available evidence suggests that conquest of the surrounding semi-arid environment may have occurred independently in at least two of the groups of tsetse flies, namely, in the *Fusca* group (*G. longipennis*) and in the *Palpalis* group (*G. tachinoides*). In the *Morsitans* group all except *G. austeni*, which it is suggested has made a secondary return to the primitive habitat, occupy arid or semi-arid environments; whether this situation has arisen by independent invasion from the ancestral type of habitat, or whether it represents a branching from a common *Palpalis-Morsitans* stem which took place after the initial spread into semi-arid regions cannot be decided; but in view of the similarity between the male genitalia of the two groups the latter alternative is not unlikely, and so it has been represented in figure 14*b*.

* My thanks are due to Dr E. Burtt who first drew my attention to these differences in lobe size, which an extensive collection of pupae and long familiarity with the subject had enabled him to detect without having resort to mensuration.

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For lack of critical information about the physiology and behaviour of the adult tsetse little can be said about the complex of factors which occasioned and made possible the spread of populations into semi-arid regions. But the very close correlation between habitat and resistance to desiccation of the tsetse pupa suggests that pupal water balance may have been an important limiting factor. In this connexion a field experiment reported by Jackson (1945) is relevant—during the dry season of 1944 a population of *G. palpalis* was released on a seasonal stream in the habitat of *G. swynnertoni*. Individuals captured during the succeeding month and brought to the laboratory produced normal and viable pupae, but in the field no second generation flies could be found. In view of present findings there can be little doubt that this failure may be attributed to inadequate waterproofing of the *G. palpalis* pupae, and the experiment lends some support to the view that pupal water balance may be a limiting factor in the colonization of semi-arid environments.

It is clear that many aspects of the distribution of tsetse flies may be explained in terms of the resistance of pupae to desiccation. The question arises to what extent may the seasonal fluctuations in pupal mortality reported by different authors be similarly accounted for (e.g. Nash 1939; Chorley 1929; du Toit 1954). With *G. longipennis*, *G. morsitans* and *G. swynnertoni* the mean diurnal humidity needs to drop below 20 % r.h. before pupal viability is affected, and this is unlikely to occur in areas which they inhabit. So where seasonal fluctuations in pupal mortality can be demonstrated for these species the cause must be sought in terms of factors other than humidity. With the other species it seems possible that a particularly severe dry season, leading in the case of category (*a*) habitats to a drying up of free water, may adversely affect the emergence rate. Such an effect would be particularly likely to occur with category (*b*) species near the limits of their distribution, for example, with *G. pallidipes* at Shinyanga and with *G. tachinoides* in Northern Nigeria (see Nash 1939).

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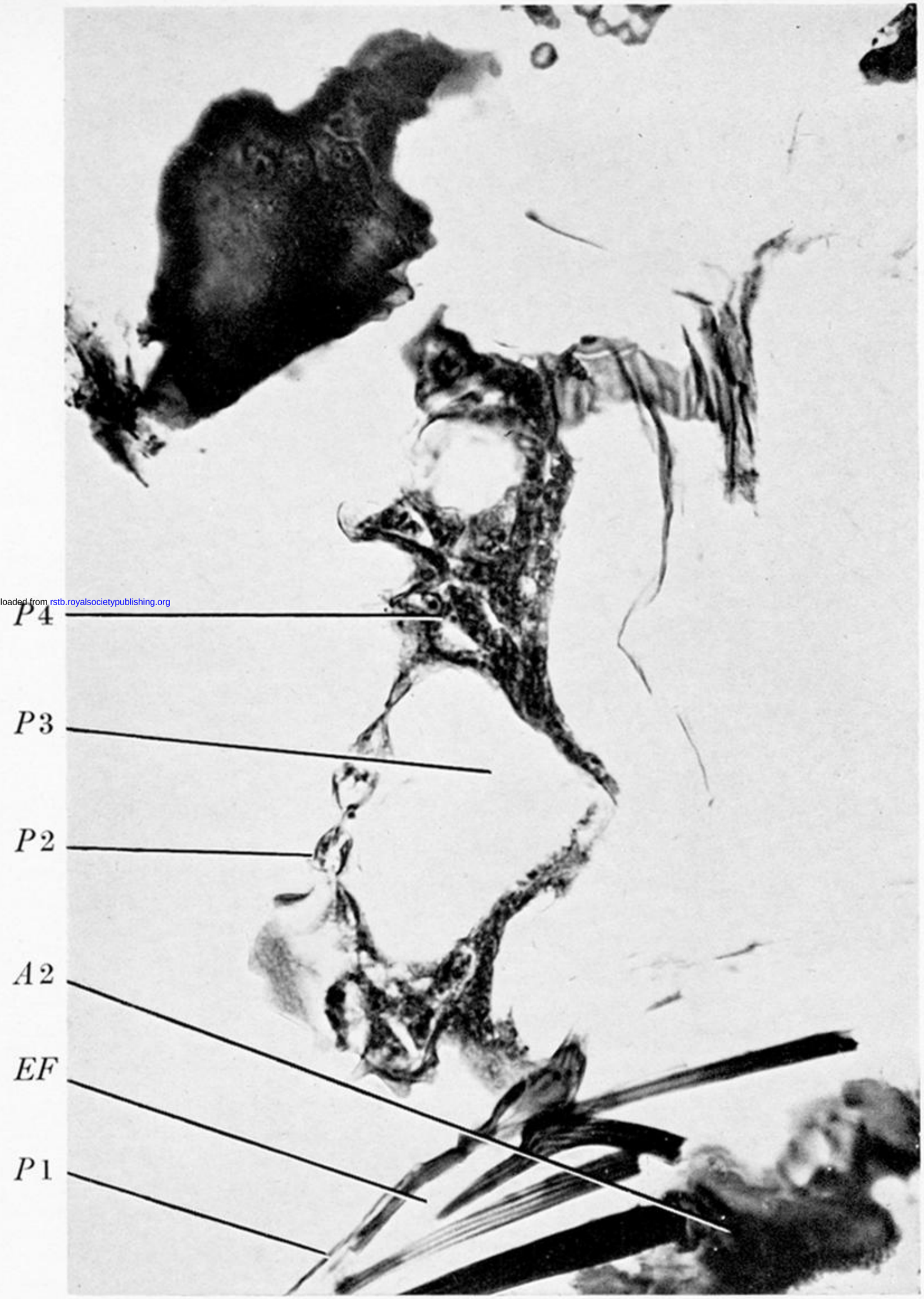

FIGURE 15. Section through the spiracle of pupa 23 days after puparium formation. *P*1, pupal skin; *P*2, pupal spiracle; *P*3, air chamber of pupal spiracle; *P*4, cells associated with pupal spiracle; *A*1, adult cuticle with spines; *EF*, ecdysial fluids.

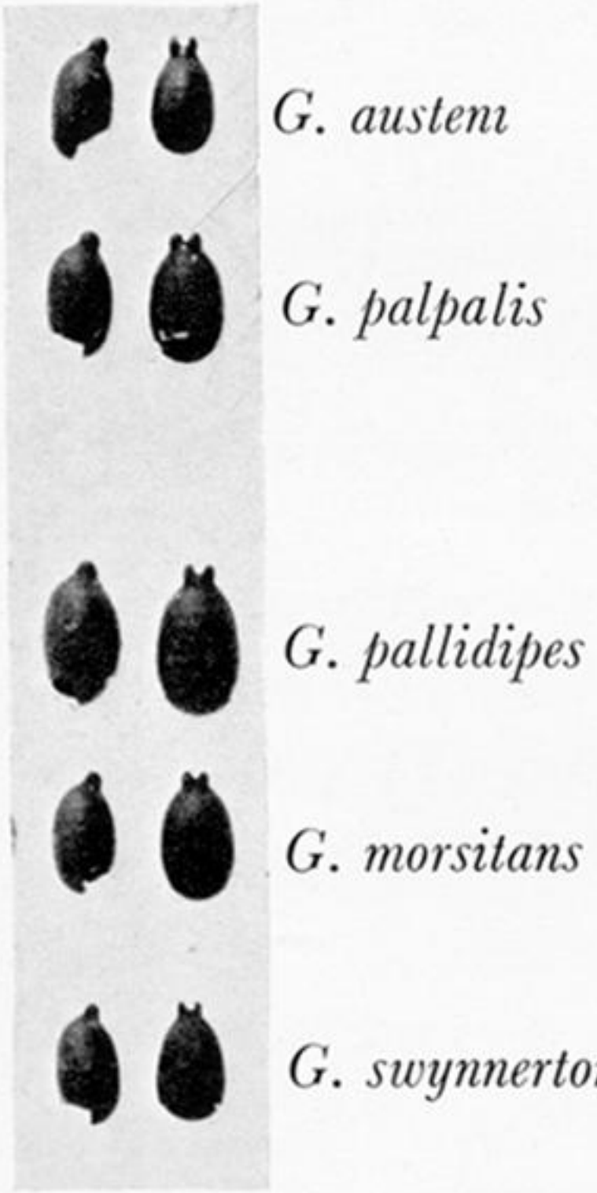

FIGURE 16. Puparia of some species of tsetse fly illustrating differences in the relative size of polypneustic lobes.

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