IV. *Further Observations on the Structure of the Scent Organs in certain male* Danaine *Butterflies.* By H. Eltringham, M.A., D.Sc., F.Z.S.

[Read December 2nd, 1914.]

Plates XI–XX.

On a former occasion * I described at some length the structure of the scent-glands and brushes in the Danaine butterfly *Amauris niavius*, Linn. I am greatly indebted to my friends Mr. W. A. Lamborn, Mr. J. C. Moulton, Lieut.-Col. Manders, Mr. S. A. Neave, Mr. C. A. Rogers, and Mr. C. A. Wiggins for further material in the form of butterflies suitably preserved, enabling me to cut sections and investigate the structure of similar glands in other species. I am also much indebted to my friends Prof. E. B. Poulton and Dr. F. A. Dixey for kindly looking over the proofs of the present paper. It may be useful to recall that in the case of *A. niavius* the scent-glands were found to be located in the patch on the hind-wing submedian nervure. Each gland consisted of several cells communicating on the upper surface of the wing with a projection which I called a "scent-cup." This structure was provided with a cover pierced in the centre by a minute pore. Each scent-cup was also protected by a small scale, differing from the normal wing-scales in size and shape.

The two brushes situated in the hinder end of the abdomen consisted of tufts of chitinous hairs † arising from trichogenic cells in a membranous sac, the eversion of which by fluid pressure causes the extrusion of the brush. In

* Trans. Ent. Soc. Lond., p. 399 *et seq.*, 1913.

† It has been pointed out to me that the insect structures commonly referred to as hairs are not hairs in the true morphological sense, and of this I am, of course, aware. At the same time, when speaking of hairs in connection with insects, we mean insect hairs or the hair-like structures (setae, modified scales, etc.), which are found on insects, and I do not think that any confusion need arise on this point.

addition to these structures it was found that a certain area of the brush-bag contained cells which produced numerous delicate chitinous filaments, these having the property of breaking up transversely into innumerable tiny particles, thus forming a kind of dust, the suggestion being that these particles assisted in the diffusion of the scent.* Reference was also made to the fact that these butterflies are known to apply their brushes to the patches on the wings.

Having thus briefly reviewed the conclusions already attained in my previous paper, I propose to describe the corresponding structures in certain other species of butterflies, and it will be seen that complicated as is the arrangement in *A. niavius*, differentiation has reached an even higher development in other forms. Neither wing-glands nor dust-producing devices are invariably present; the brush itself and not the wing may produce the scent material, or at least exhibit a glandular structure, whilst the dust may be produced by the wing and not by the brush, and in the pupal instead of in the imaginal state.

I propose to describe each species separately, after which the results may be considered.

Amauris psyttalea, Plötz.

Of this species I have had only one body to examine. It was sent to me by Mr. C. A. Wiggins, who did not then understand that I required the wings as well. I am thus unable to describe the wing-brands except from such information as can be gathered from the examination of dried examples. From these, however, it is clear that the arrangement is very similar to that in *A. niavius*. Regular rows of "scent-cups" are seen, each covered by a small scale arising from a socket placed between two of the scent-cups in the adjacent row. The scales resemble those in *niavius*, though they are slightly less rounded. The glands are probably similar to those in *niavius*. From the single abdomen available I was able to make both transverse and longitudinal sections of the brushes, and these proved extremely interesting. From the base of the brush there arises a pencil of hairs corresponding in

* This idea of the function of the dust particles was in part due to the suggestion of my friend Dr. Karl Jordan, a fact which I regret I omitted to acknowledge in my previous paper.

every way with the "yellow hairs" * in the brush of *niavius*. Pl. XI, fig. 1, is a photograph of a section of the brush near the base. It will be noted that there are a few small dark-coloured hairs near the middle. These resemble similar hairs in the brush of *A. whytei*, but they are fewer in number and much shorter. A little further from the base the brush-bag becomes deeply convoluted on its inner side and bears great numbers of small cells, from which arise delicate filaments similar to the "dust-hairs" in *A. niavius*. Pl. XI, fig. 2, is a photograph of a section across this area. In the latter species these dust-hairs, though numerous, are almost insignificant compared with their development in *psyttalea*. In the latter they form the most conspicuous feature of the brush, and are produced in enormous quantities. They arise from cells all round the brush-bag, and in section they form a dense ring enclosing the yellow pencil. Pl. XI, figs. 3 and 4, show the appearance of sections of the brush near the middle, and towards the outer end, respectively. The dust-hairs extend to the end of the brush, where they show the same tendency to break up into particles. From an area some little distance from the basal end of the bag there arise the black hairs similar to those in *niavius*. These can be seen in figs. 3 and 4.

The prolific production of dust material in *A. psyttalea*, as shown by microscopic examination, is interesting when considered in conjunction with Dr. Carpenter's observations on the courtship of the species. Dr. Carpenter's note was read by Prof. Poulton at the meeting of the Society on December 2nd, 1914, at which I exhibited photographs of sections of Danaine scent-organs illustrating the present paper. His remarks were as follows :—

"On July 21 [1914] at the edge of the forest here on Kome Island, about 5 p.m. I saw the courtship of *Amauris psyttalea*, Plötz. I noticed two flying about, obviously a male pursuing a female. Presently the latter settled on an erect dead flower-spike of an aromatic Labiate, about two feet above the ground. She sat with head upwards and body perpendicular, wings outspread at right angles.

* I have referred throughout to these hairs as the "yellow hairs," since they are of that colour in all my preserved specimens. As Dr. Carpenter, in the observation quoted below, speaks of them as "quite white" in the living insect, I conclude that they become yellow after immersion in preservatives.

The male hovered flutteringly about four inches over her head, rising and falling a little, but on the whole at about the same level. His abdomen hung down a little, and every now and then at intervals of a few seconds the two flaps [the male claspers, especially large in Danaines] at the end of the body were widely separated (so as to stand out at right angles to the long axis of the body), and the brush was quickly protruded and as quickly drawn in again. I was surprised to see what a large structure it was, being quite white and visible at a distance of several yards. In fact, I first noticed it at that distance and went closer to see what was going on. The female sat quite still except for an occasional slight movement of the wings. I watched for a minute or so, and it was impossible to doubt that the male was endeavouring to excite the female. Just as I thought I would catch them as records, the female suddenly flew away and the male followed. I have, however, no doubt of the species.

"The very sudden protrusion of the brush might easily cause the peculiar fine hairs of stellate section, described by Eltringham, to break into sections which would float like dust in the air."

There can be no doubt that the dust material plays an important part in the courtship of these insects. Pl. XI, fig. 5, is a photograph of a very small area of a slide which has been dusted with a brush taken from a dry specimen. The filaments which form the dust are very similar to those found in *A. niavius*, but in transverse section whilst presenting an irregular outline, appear less distinctly stellate. They arise from very deep sockets, and communicate with their respective hypodermal cells through fine canals which traverse the chitinous membrane of the bag. They seem not to break into such very short pieces as the *niavius* filaments.

Amauris egialea, Cram.

The structure of the wing-brands in this species differs in several details from that found in *A. niavius*. Pl. XVIII, fig. 3, shows the shape of the normal scales as compared with those found on the scent-patch. The latter, one of which is shown in the figure, are considerably elongated instead of rounded as in *niavius*; moreover, they do not closely cover the glandular area. Pl. XVIII, fig. 4, shows a semidiagrammatic view of a portion of the brand viewed

from above, from which it will be seen that the rows of scent-cups, indicated by dotted circles, are separated by glandular areas without cups, whereas in *niavius* there were no such intermediate rows. As a result of this formation, a transverse section of the scent-patch taken parallel with the general direction of the nervures has a quite different appearance from a section taken at right angles to this direction. Pl. XVIII, fig. 1, shows a section parallel with the nervures. On the upper wing surface the scent-cups are seen, each with its gland consisting of a few more or less vacuolated cells with prominent nuclei. The scales are rather thick and apparently solid. It should be noted that the glandular cells are short and do not reach to the ventral or lower wing-surface, thus leaving a space which is possibly filled with fluid during life. Alternating with these cupped glands are what may be termed " blind glands," formed of cells resting on a mass of material which is preserved in the section and presents a horizontally striated appearance. That this is really a mass of material, and not a membrane, is proved by the fact that its appearance is continuous in consecutive sections in whatever direction they may be cut. It presents the same appearance in sections cut in planes at right angles to each other.

Pl. XVIII, fig. 2, shows a section transverse to the nervures and through a row of the cupped glands. The difference in wing thickness is due to the section having been taken nearer to the edge of the patch. Also, the glands being somewhat flattened, they appear broader in this section. In all other respects the structure of the glands appears to be the same as in fig. 1.

The abdominal brushes are situated as in *A. niavius*, and are similar in so far as they consist of chitinous hairs arising from the lining of a membranous bag. They are everted by fluid pressure in the body cavity of the insect, and withdrawn by means of a muscle attached to the proximal end of the bag and to one of the ventral abdominal plates. The structure of the brush is, however, very different. When dissected out it is found to be of a fairly uniform thickness up to within about 1 mm. of the proximal end, where it is somewhat sharply constricted and, for the remainder of its length, considerably narrowed. From this constricted portion there arise structures which are very different from the well-rounded hairs more commonly found in these brushes. They are presumably in

a more primitive condition, resembling thick scales of irregular section and comparatively great length. They arise from cells with chitinous sockets quite similar in appearance to those giving rise to the other hairs or scales, but above the stalk they are abruptly expanded so that their mean diameter is much greater than that of the sockets from which they arise. The base of the brush is composed of these structures only, and in the retracted condition they form a central cone, lying in the heart of the brush and ending in a point at about two-thirds of the distance from the base of the brush to its extremity. These bodies readily take stains such as haematoxylin and carmine, and are not chitinised to anything like the same extent as other portions of the brush. Pl. XVIII, fig. 5, shows a section of one of these structures as seen under a very high power. It is evident that the shape is such as to increase as much as possible the surface area. Moreover, I am inclined to think, after careful examination, that numerous pores exist in the surface, so that it is reasonable to suppose either that some secretion is discharged therefrom, or that they serve as a store for the secretion extracted from the wing-glands. There is, however, no direct evidence of their function.

Pl. XII, fig. 1, is a photograph of a section of the brush, where it consists solely of these structures. The membrane of the brush-bag is here very thin and contains many small nuclei. Pl. XII, fig. 2, shows a section taken a little above the constricted portion of the brush. Here the outer membrane has become thicker, and the large nuclei are those of the ordinary hair-producing cells. Two new structures are now seen to have arisen.

(1) Forming a ring round the central bodies already described are structures whose walls are evidently thin, and present in section an irregular and considerably convoluted appearance. These bodies enclose and thicken the cone in the heart of the brush, dying out to a point a little nearer its distal end. A highly magnified section of one of these is shown on Pl. XVIII, fig. 6. Inside the thin wall is an exceedingly delicate tissue with distinct dots scattered through its substance, and showing a more or less radiate striation. The walls of these bodies do not show signs of the presence of pores.

(2) Outside these " first-ring " bodies we see the ordinary brush-hairs—not, at this level, very well rounded in sec-

tion, but still recognisable as corresponding to the hairs in the *niavius* brush. Pl. XII, fig. 3, is a section a little further towards the distal end. Here the central bodies occupy a reduced area. They are still surrounded by the first-ring bodies, but what may now be called the normal hairs occupy a greater space. It should be particularly noted in this figure that the outer layer of the normal hairs includes relatively many having a flattened section. Pl. XII, fig. 4, is a section still further outwards. The central bodies have almost come to a point, and are surrounded by a fairly thick mass of first-ring structures. The normal hairs, or "second-ring," occupying a large area, are those of the yellow tuft of the brush, corresponding with the yellow hairs of *niavius*. One of these hairs is shown in section at Pl. XVIII, fig. 7. The central lumen is small, the medullary substance presents a more or less radially striate appearance, and the outer surface is longitudinally ribbed.

(3) The black hairs have now begun to arise from the bag membrane. They are of variable diameter, and are separated from the yellow hairs by those of flattened section which occupied the extreme outer layer in fig. 3. We may call these the third-ring bodies. In Pl. XII, fig. 5, the central cone has finally disappeared, the first-ring bodies are coming to a point, whilst the normal hairs, yellow and black, occupy nearly the whole of the area. In Pl. XII, fig. 6 the normal hairs remain, divided, however, by the third-ring bodies, which are larger and flattened towards the end of the brush, and are here seen to be distinctly differentiated. In some species the hairs which thus become terminally flattened eventually break up into small fragments, and thus provide the "dust" which so frequently occurs in connection with these organs.

In *A. egialea* this breaking up does not seem to occur, nor do any of my sections show that dust is produced at all in this species.

The structure of the organs in *A. egialea* differs from that in *A. niavius* in the following manner. The cupped glands of the wing-patch are not continuous, but have rows of blind glands between them. The scales are of a different shape, and do not provide a complete protection for the cups. The gland-cells do not occupy the whole space between the wing-membranes, and those of the blind glands rest on a substructure which presents a horizontally

striated appearance. The brush does not consist solely of the yellow and black hairs, but is provided in addition with a core of curiously modified structures of which those occupying the centre are but little chitinised, whilst the yellow and black hairs are separated by a layer of bodies of irregular section extending to the outer end of the brush, where they become much flattened. There is apparently no apparatus for producing dust.

Amauris ochlea, Boisd.

In this species the wing-patch, whilst differing slightly from that of *A. egialea*, resembles it closely in its main features. The scales of the wing-brand are very similar in shape, and in the same way they fail to cover the glands. The cupped glands are in rows which, as in *egialea*, alternate with rows of blind glands. In section the appearance is also similar, but though the gland-cells are of about the same size, the whole wing-patch is somewhat thinner; hence in the cupped glands there is much less space between the cells and the underside of the wing; whilst beneath the cells of the blind glands there is but a trace of the horizontally striated tissue.

The abdominal brushes of *A. ochlea* are also very similar in structure to those of *egialea*. There is a core of central bodies surrounded by first-ring structures, and the black and yellow hairs are separated by a layer of third-ring bodies. These latter, however, are flatter near their origin, after which they become more rounded, and finally again flattened towards their extremities. These bodies, together with the yellow hairs, have, however, a different structure from those of *egialea*. Instead of being regularly striated their surface is studded by immense numbers of what may be termed chitinous nodules, which are very conspicuous both in transverse and longitudinal section, and towards the distal end of the brush undoubtedly become detached, forming a kind of dust which serves the same purpose as the dust produced in various ways by other species. Pl. XVIII, fig. 8, shows a transverse section of one of the hairs, whilst fig. 9 shows the appearance in a longitudinal view. Not only is the outer surface covered with nodules, but similar particles appear to be formed within the hair. It may be noted that these nodules are rather readily stained by haematoxylin. They

are not the dried granules of a secretion, since they are unaffected by prolonged immersion in eau-de-javelle, although particles of secretions which I have observed in other species, however fully they may resist the action of the various solvents used in preparing the sections, disappear more or less readily when a drop of eau-de-javelle is placed on the slide. The dust produced in this manner in *A. ochlea* must be very small in amount as compared with that formed in such species as *niavius* and *psyttalea*. I have not reproduced photographs of sections of the brush in *A. ochlea*, nor drawings of the wing-glands, since compared with the same organs in *A. egialea* the differences are so small as scarcely to warrant separate illustration.

Amauris hecate, Butl.

The wing-patch presents alternate rows of cupped and blind glands, as may be seen in Pl. XVIII, fig. 12, showing a section parallel with the nervures. The gland-cells differ considerably from those of *A. egialea*, in that they occupy nearly the whole space between the wing-membranes. The structure may be regarded as somewhat intermediate between that of *niavius* and *egialea*. The scales on the wing-patch, of which one is shown in fig. 10, are also intermediate in shape. They cover the glands very incompletely, though more effectively than those of *egialea*.

The structure of the brush in *A. hecate* is also of an intermediate character. Pl. XIII, fig. 1, shows a section near the base, from which it may be seen that there is a small core of modified scales presenting in large part the characters of both the central and first-ring bodies of *egialea*. These die out rapidly and the greater part of the area of the section is taken up by the yellow hairs, as shown in Pl. XIII, fig. 2, in which will also be noticed, on the side, where the bag membrane is thinnest, a somewhat obscure mass, unfortunately not very clearly in focus. This represents certain flattened hairs which arise on that side and become at higher levels (figs. 3 and 4) somewhat invaginated on one side so as to present a reniform section. These structures may perhaps be regarded as corresponding with the third-ring bodies of *egialea*. In fig. 4 they occupy a large area of the section and are obviously tending to break up, a condition shown still better in fig. 5. They arise from cells in the bag membrane, and in longitudinal

section are not at first distinguishable from the yellow hairs. They soon, however, begin to present a nodulated appearance similar to that in *ochlea*, and their disintegration towards the distal end of the brush produces a considerable quantity of dust corresponding with that of other species. It will be noticed from the sections that the black hairs in this species are of comparatively small diameter, and that they arise from an area of the brush-bag which is greatly thickened by the aggregation of small trichogenic cells. Pl. XIII, fig. 6, is a longitudinal section of this area. Pl. XVIII, fig. 13, shows a section of a yellow, and of a black hair, and fig. 11 represents the appearance of one of the flattened and nodulated hairs which ultimately break up to form the dust material. This disintegration occurs through the gradual decrease in the lumen and the thinning down of the bases of the nodules, until the latter become detached. A longitudinal view of one of these hairs presents much the same appearance as that of the corresponding structure in *A. ochlea*.

Amauris whytei, Butl.

The wing-patch in this species is provided with scales which closely resemble those of *A. egialea*, both in shape and in the fact that they do not form a continuous protection for the upper surface of the glands. The structure of the wing-patch differs from all those previously described in not showing any definite differentiation into separate glands. The cup-like structures occur in fairly definite rows, separated by rather wide spaces representing the blind-gland areas described in other species. It will be seen from the section shown on Pl. XIX, fig. 14, that the glandular area is very thin and that the gland-cells are rather irregularly placed between the wing-membranes. Strands of connective tissue joining the two surfaces of the wing presumably exist, although they are but little evident. In this species the wing-patch is either in a more primitive condition or is, so far as thickness and differentiation of structure are concerned, degenerate.

The abdominal brushes are also in some respects different from those already described. Pl. XIV, fig. 1, shows a section of the brush near its base. The brush-bag is seen to be comparatively thick. Large hair-producing cells are present, and in at least two instances the central lumen of

these is plainly visible. The first to arise are the small dark-coloured hairs seen near the middle of the section. These form a core continued throughout the length of the brush, and, unlike the components of the core in the *egialea* brush, are heavily chitinised from their origin. Surrounding these are hairs of large diameter, less chitinised, and corresponding with the yellow hairs of other brushes described. The remainder of the section is seen to be occupied by hairs of relatively small diameter and less distinct outline. These at their origin are scarcely distinguishable from the yellow hairs, though they soon become differentiated by their granular appearance. In Pl. XIV, fig. 2, the black hairs have begun to appear at one side and the cuticle of the brush has become thin. Figs. 3 and 4, representing the same structures at higher levels, show an increasing diameter in the hairs of the core. In fig. 5, still higher, the black hairs have become less numerous and those of the core smaller, whilst the granulated hairs now betray their purpose as dust producers, breaking up and forming small nodules as in *A. ochlea*. Fig. 6 is a section taken still nearer the end of the brush. The black hairs have nearly died out, the core hairs are much reduced in diameter, and the dust hairs are continuing to disintegrate. On Pl. XIX, fig. 15, I have endeavoured to show the appearance of one of the yellow hairs as seen under a high power. The outline is very irregular, and the wall seems to be pierced by minute pores occurring here and there, not on the longitudinal ribs, but between them. I am not really certain of the existence of these pores, but careful examination of sections strongly suggests their presence. On Pl. XIX, fig. 16, I have shown one of the granulated hairs as it appears when breaking up. In a section taken near the end of the brush the whole field is strewn with the separated particles.

The principal peculiarities of the scent-organs in *A. whytei* are, then, the thin and simplified wing-patch, and the central pencil of fine stiff hairs in the brush.

So far the organs examined have all been taken from species of one genus, viz. *Amauris*. It will now be profitable to investigate the structure of the scent-apparatus in other Danaine butterflies.

As an example of a small but important genus we may take **Tirumala petiverana,** Doubl.

In this genus the alar scent-organs do not form a patch,

but consist of a fold or pocket of the wing. As the brushes are of a less complicated structure than the scent-pocket, it will be convenient to describe them first. They consist of the usual membranous bag from which arise the hairs of the brush, which in this species are all of one form and strongly chitinised. It is unnecessary to give a photograph of more than one section of the brush. Pl. XV, fig. 4, represents the general appearance of any transverse section of that organ, whilst Pl. XIX, fig. 23, shows one of the hairs in section as seen under a high power. I am inclined to think that these hairs are pierced by minute pores. At any rate a very thin section shows a number of pale radial lines between the low ridges which longitudinally traverse the surface. Within the hair-wall is a delicate medullary substance pierced by a longitudinal canal of irregular section, and often of very eccentric position. Pl. XIX, fig. 21, shows a longitudinal surface view of one of these hairs. The structure is very faint, and only visible under a high power, which shows that the fluting or ribbing is not, at least, in the greater part of the hair parallel with the long axis, but takes an undulating course. With regard to the presence of pores in these hairs, it is curious that their existence should be rather strongly suggested in a species in which their utility is least obvious, for the production of a scent-secretion as well as of a dust material appears, as will be seen later, to be amply provided for by the wing-pocket. One other feature remains to be noted. A considerable portion of the interior of the brush-bag, especially towards the open or distal end, is not smooth as in most of the other species examined, but thrown into a great number of minute irregular folds, giving it on a surface view the appearance of being covered by a mass of tiny excrescences. This feature, which is not accompanied by any glandular development, occurs also in *Danaida chrysippus*. So far its significance is obscure.

Pl. XV, fig. 5, is a photograph of a section of the scent-pocket in *T. petiverana*. It consists of an outer chitinous layer bearing large scales, and morphologically part of the underside of the wing. Next to this is a layer of irregular cells, followed by a second layer, from which arise the small dark scales seen in the photograph. Within the cavity of the pocket masses of thread-like bodies may be seen lying loose. Pl. XIX, fig. 19, shows more accurately the structure

of the various layers of the pocket. The inner layer exhibits numerous vacuolated cells more or less separated by strands of chitinous material, and associated with scale-sockets from which arise the scent-scales. These are in the form of flattened elongated bags, having a central cavity which almost certainly opens by a pore at the outer end of the scale. Beneath this layer is a second stratum of cells also showing large vacuoles. This layer is highly developed at the closed end of the pocket and becomes greatly attenuated towards the mouth, where it opens on the upper surface of the wing.

It seems convenient here to refer to the notes of Mr. J. C. Kershaw on the structure of the scent-pocket in the very closely allied species *T. limniace*, occurring in the Oriental Region. Mr. Kershaw made, but did not publish, numerous observations on the scent-glands and genital apparatus in certain Oriental Danaines, together with some excellent line drawings. The notes and drawings were handed over to Prof. Poulton at Oxford, and I have his and Mr. Kershaw's kind permission to make use of them. Speaking of *limniace* he says :—

" The interior of the pocket or sac on the underside of the hind-wing (with the opening thereto on the upper side of the wing) is lined with scales entirely different to those overlying the rest of the wing. These scales are bottle-shaped and filled with fluid, but I was unable to detect any special scent. Many of these scales are reduced to powder which lies in the cavity of the sac."

In a subsequent note the same author says :—

" The oily nature of the scent-scales, or rather the oily nature of the liquid contents of the flask-shaped scent-scales, is easily seen when a batch of scales is scraped off and crushed on a slip under a moderate power; or if a single scale is crushed with a needle under a fairly high power, especially if a drop of water is placed on the slip, when the liquid from the scent-scales behaves exactly like oil, floating on the surface in minute globules. The scent-scale liquid is also greasy to the touch, but, of course, a large batch of scales must be crushed to get enough material to test by touch."

The above observations were, of course, made on fresh examples, and my specimens, which had been treated with preservatives and then kept in spirit, do not show the oily contents of the scales. Mr. Kershaw's notes are therefore

of the greatest value in enabling us to learn something of the function of the scent-pocket. We may, I think, fairly assume that the vacuolated cells form the glands which produce the oily secretion, and that this is accumulated in the scales and discharged thence into the wing-pocket. There remains then the dust material which occurs in such large masses in the pocket, and can also be observed entangled in the hairs of the anal brush. I was for some time at a loss to discover the true origin of the dust filaments. In my sections of the scent-pocket they were invariably found loose in the cavity and already much broken up. I found the same substance, but in a still more disintegrated condition, amongst the hairs of the abdominal brush. In the brush there was no evidence of dust production, whilst its presence in larger quantities and in a less pulverised condition in the scent-pocket strongly suggested the latter as its place of origin. Probably the truth of the matter would still have remained a mystery, but for my having been fortunate enough to secure, through the kindness of my friend Prof. Poulton, a pupa of *T. limniace*, the oriental form of this species, in which the imago was almost completely formed. The specimen had been sent to the Hope Department from Ceylon by Lieut.-Col. N. Manders. The pupa was not preserved in any way, being merely dried, and I had small hope that sections of any value could be cut from it, but the inspiring optimism of my friend above mentioned was quite justified. A prolonged evaporative embedding in celloidin resulted in my being able to cut sections which revealed the highly interesting fact that the dust filaments are produced beneath the pupal covering, and that, by the time emergence takes place, the cells from which they arose have become practically atrophied. The examination also showed that up to the time of emergence there is *no scent-pocket at all.* The area which subsequently becomes a pocket is, as in the *Amauris* imago, a patch on the hind-wing. In *Tirumala*, however, the expansion of the wing after emergence causes an invagination of the patch, which thus becomes a pocket. In dissecting out the wing from the dried pupa already mentioned I found that the patch was covered with a rather thick mass of material, which in its dry state scaled off and fell to pieces at the least touch. This substance mounted in clove oil proved, as was expected, to

be entirely composed of the dust filaments, some of them being of surprising length. They break with such facility, and are so inextricably interwoven, that it was impossible to separate out a single filament. Pl. XV, fig. 6, shows a photograph of a few particles lying at the edge of a mass of the material, whilst Pl. XIX, fig. 22, shows small pieces of the filaments as seen under a high power. Pl. XIX, fig. 20, shows a section of the dried wing before emergence from the pupa. Cell structure is of course absent, as the soft parts have not been preserved, but sufficient remains to show that in the unexpanded wing, the scent-patch, as it then is, contains cells which produce the filaments, forming a mass of dust material, the latter becoming enclosed in the pouch as it is formed by invagination. The little filaments shown in fig. 20 are merely the unbroken basal ends of threads of much greater length. Careful examination of many sections leads me to suppose that the cells which produce these filaments alternate with those which give rise to the scent-scales. The thread-producing cells cannot be recognised in the imaginal wing, and I can only suppose that, having produced the dust material, their function ceases and they become crowded out by the increased development of the vacuolated oil-producing cells.

In *T. petiverana* we have therefore a curious contrast with the structure in *A. niavius*. Instead of the dust material being produced in the brush, it is formed by the wing-patch, although only by that organ in the pupal state.

Danaida chrysippus, Linn.

So much is now known of the bionomics of this abundant species that it is interesting to be able to add something to a knowledge of its structure. The male possesses a small scent-pocket in the hind-wing and the usual pair of abdominal brushes. The scent-pocket is not formed quite like that of *T. petiverana*, since it is really a projection from the wing surface subsequently folded over to form a kind of recess. Thanks to my friend Lieut.-Col. Manders, I have been able to make preparations from the pupa of this species, sent to me in a properly preserved condition, so that something of the final development of the scent-organs has been disclosed.

The brush contains hairs of one kind only and of simple

structure. Both transverse and longitudinal sections show that there are, entangled in the hairs, large numbers of minute spherules—apparently a coagulated secretion. They are certainly not chitinous particles of dust material, since they dissolve fairly readily and not slowly in eau-de-javelle. From the appearance of these granules in a longitudinal section, one is tempted to believe that they are extruded from the hairs themselves, since they adhere thereto very closely in many places. The absence, however, of any glandular structure in the cells of the brush-bag lends greater support to the theory that they are in fact a product of the secretion produced in the wing-pocket. The brush-hairs are round in section for part of their length, but become much flattened towards their distal extremities. Pl. XIX, fig. 26, shows a section of one of these hairs; fig. 27 a longitudinal surface view. The surface of the hair is seen to be traversed by longitudinal curved ridges, each of which bears two rows of small projections arranged alternately on each side of the crest. As in *T. petiverana* there is no pocket in the unexpanded wing, but the portion which afterwards becomes invaginated consists of a diverticulum. Pl. XVI, fig. 1, is a photograph of a section of a wing before emergence. On the right may be seen the outgrowth from the wing-surface covered on its outer side by a layer of small thick scales. The double row of delicate structures in the centre of the section represents the ordinary scales of the upper surface of the wing. Pl. XX, fig. 28, is a drawing of a small portion of that part of the unexpanded wing which will form the scent-pocket in the imago. The cells which produce scales are highly vacuolated, and alternate with cells attached to cup-like projections somewhat similar to those already described in other species. The cup-cells at this stage seem mainly to produce those long processes described by Mayer,* which ultimately form the fibres holding together the upper and lower surfaces of the wing during expansion. Nevertheless some of them ultimately develop into cells which appear to differ but little from those attached to the scale-sockets. The cause of the folding in of the diverticulum during expansion may be easily discerned by comparison with Pl. XIX, fig. 25, which shows in section a part of the ordinary unexpanded wing-membrane. Here the chitin is thrown into deep folds which

* Bull. Harvard Mus., vol. xxix, p. 209 *et seq.*, 1896.

will become flattened during expansion. The patch is already flat, and being incapable of extension, becomes covered by a fold. Pl. XIX, fig. 24, shows a cup and a socket-cell from the imaginal wing. These cells are highly vacuolated, finely granular, and occasionally provided with more than one nucleus, especially the socket-cells. Mayer (*loc. cit.*) describes the nuclei of the scale-producing cells in the wing of *D. plexippus* as occasionally undergoing amitotic division, so that we may suppose this process also to take place in the present species (a broken scale is shown arising from the right-hand cell). The "cups" have no relic of a scale-stalk as in *D. lotis*, but appear to possess a central pore, though this is scarcely so obvious as in some species of *Amauris*. The scales lining the pocket have an appearance which suggests that they are traversed by pores, though the structure is less easily discernible than in *lotis*. There is no evidence in either pocket or brush of the production of any dust material.

A longitudinal section of the brush within the pupa shows the hairs arising from highly vacuolated cells, each with a large nucleus. The cytoplasm is finely granular, and stains less readily towards the extremity from which the hair arises.

Danaida lotis, Cram.

In this species there is a fold in the hind-wing resembling that of *D. chrysippus*, and a pair of abdominal brushes, the latter presenting no special features. The hairs are of one kind and strongly chitinised. In general their sections resemble those of *chrysippus*, and small spherical particles of a dried secretion may be observed entangled amongst them. Some hairs are flattened for a portion of their length or depart in other ways from the more truly circular section usually exhibited. Their functions are probably merely mechanical. Pl. XVI, fig. 2, is a photograph of a section of the brush not far from the distal end. Pl. XVI, fig. 3, shows a photograph of a transverse section of the wing-pocket, which is seen to be a more or less spiral fold of the wing. The pocket contains no dust material, and as none is found in the brush it is evidently not produced in this species. A surface view of the membrane with which the pocket is lined shows that alternate scale-sockets are modified into structures resembling scent-cups, whilst the remaining sockets present a more or less normal

appearance. A section through a portion of the pocket is shown at Pl. XX, fig. 29. The cupped glands contain a comparatively small cell, finely granular and possessing a large nucleus. From each arises a delicate projection which is doubtless a degenerate scale. From each unmodified socket arises a scale, and they are sufficiently numerous to form a complete lining to the pocket. These scales are porous, the minute openings lying along the striations of the surface mainly in the central area of the upper side. In connection with each scale is a large cell, the upper part of which is densely packed with large and conspicuous granules having a deep brown colour in unstained examples. These coarse granules usually obscure the nucleus which is also present in the upper half of the cell. They do not dissolve in ether, alcohol, or xylol, and are only very slowly affected by eau-de-javelle. The remainder of the cell is occupied by a delicate and highly vacuolated reticulum, presenting a finely granulated structure. Whether the hair-like vestigial scales arising from the cupped cells are open tubes or not I have been unable to decide. The covers of the cups have, however, rather the appearance of being minutely perforated, though this point is also extremely difficult to decide.

It should be noted that the structure described above, in so far as it consists of special scales alternating with delicate stalks, is similar to that described by Müller as occurring in *D. erippus*.*

Parantica eryx, Fab.

This species bears on the hind-wing a scent-patch of considerable size and presenting a somewhat pear-shaped outline. In surface view it shows regular rows of scent-cups alternating with normal scale-sockets from which arise scales much resembling those of *Amauris niavius* in shape, though rather larger. They form a continuous covering for the scent-patch of which a section is shown at Pl. XIX, fig. 17. Perhaps the most striking features of the patch are its comparative thinness and the large number of nuclei observed in each gland. In some cases I have counted as many as seven. Whether this number indicates the presence of seven cells, or whether some cells have more than one nucleus, has not been determined. There

* See my previous paper, Trans. Ent. Soc. Lond., p. 403, 1913.

are no intermediate or blind glands. The portion of the patch from which the drawing is made represents its maximum thickness. A considerable area is not any thicker than the rest of the wing, the upper and lower membranes being in close juxtaposition, and the glands so reduced as to occupy merely the interior of the scent-cup projecting from the upper-wing surface. The brush is of simple structure containing but one kind of hair. Pl. XV, fig. 1, is a reproduction from a photograph of a section of the brush near the base of the bag, showing that the sections of the outer hairs are not circular but irregular in outline. Fig. 2 represents a section taken at about the middle of the brush, and fig. 3 one near the end. In the latter it will be observed that the hairs have rather obscured outlines, due to the fact that they produce a certain amount of dust material by the breaking off from their surface of small projections. Pl. XIX, fig. 18, shows a longitudinal view of a portion of one of the hairs bearing small leaf-like projections which readily break off, and thus form detached particles. The dust produced is of very small volume.

Trepsichrois mulciber, Hubn.*

The scent-organs in this species consists of a pair of abdominal brushes and perhaps a large patch of special scales near the costa of the hind-wing, though in the case of the latter there is some reason to suppose that, if it has to do with the scent-apparatus at all, the function is mechanical. As compared with the brushes in the species already described, those of *T. mulciber* differ in their distinct connection with the actual seat of production of the scent. The brush-bag differs from that of the only other related species which I have been able to examine, in being provided with a peculiar accessory vessel at its base, though I would at once state that I have unfortunately had only a single example from which to make preparations, and the possibility, however remote, must be borne in mind that this structure may have been an abnormality. When dissecting out the brushes this accessory vessel became visible to the eye and appeared as a small vesicle attached to the proximal end. Pl. XVI, fig. 4, represents a photograph of a longitudinal section passing through the vesicle; fig. 5 a transverse section at the point where the vesicle

* Better known as *Euploea midamus*.

opens into the base of the brush-bag proper, in which latter a few of the hairs can be seen cut across. Fig. 4 shows that the vesicle consists of an outer layer of cells with conspicuous nuclei. From these cells arise narrow funnel-like structures somewhat chitinised and homologous with hair-sockets. From these, again, spring very delicate tubes of irregular section which more or less fill the cavity. Pl. XX, fig. 31, shows one of these funnel-sockets arising from the deep layer of vacuolated cells, the latter being partly seen in section. The delicate tube arising from the socket bulges out and overhangs the latter. The socket shows a core of cytoplasm which, judging from stained preparations, appears to divide at the distal end. The whole structure strongly suggests that some secretion is discharged from the funnel-sockets into the vesicle, through the delicate tubes, and passes thence into the cavity of the brush-bag proper. Pl. XVI, fig. 6, represents a photograph of a longitudinal, and Pl. XVII, fig. 2, a transverse, section of the brush-bag. The latter is distinctly glandular, many more cells being present than are found in species of *Amauris*. The hair-sockets are long and more or less funnel-shaped as in the vesicle, though somewhat less constricted at the middle of their length. Pl. XX, fig. 30, shows one of the hair-sockets under a higher power. Several cells are seen to form a basal gland in connection with a single hair, and at one side appears the lumen of a trachea, many of which are found in intimate connection with the brush-bag in this species. Pl. XX, fig. 32, shows a transverse section of one of the brush-hairs at a point towards its extremity, whilst fig. 33 represents the appearance of a longitudinal view of the same portion of the same hair. Near their origin and for a considerable portion of their length these hairs are either comparatively smooth or only faintly striated, but towards the distal extremity they develop more of less regular lines of projections. I have been unable actually to see pores in these hairs, but that there are openings to the exterior seems a reasonable conclusion from the glandular nature of the brush-bag. One other point should be noticed. The wall of the bag is glandular and gives rise to hairs along nearly its whole length, so that the appearance on eversion must be that of a test-tube-brush instead of a spherical tuft as in *Amauris*. I have found no evidence of any dust-apparatus in this species.

We have now to consider the special area on the hind-wing. On the upper side, extending over a large portion of the discoidal cell, and from the third median to the sub-costal is an area covered by long scales which give it a furry appearance. One of these scales is shown at Pl. XX, fig. 34*a*. It consists of a long narrow section culminating distally in a spatulate formation which bears numerous small hair-like projections. In the discoidal cell of the hind-wing there is in addition a somewhat quadrate patch of greyish brown scales, which also differ from those covering the rest of the surface. One of these scales, also shown at Pl. XX, fig. 34*b*, is seen to be broad, with a length of about three times the width, and apparently somewhat thickened in the central area.

The function of these specialised scales is obscure. They are supposed to be, and possibly are, scent-scales, though sections through the wing at points where they occur do not show any special cells, or indeed any cells at all, in connection therewith. If the insect can by any means transfer scent material from its brushes to the fur patch, the latter might serve as a distributing apparatus, but we require greater knowledge of the living butterfly in its natural surroundings before the function of these patches can be established. Kershaw mentions the species in the notes above referred to, and of the use of the brushes he speaks as follows :—

" Whilst flying up and down some shady pathway the butterfly often everts and retracts the glands, sometimes partially and sometimes fully : both when solitary and when in company with others of its kind. I could never be quite certain that the glands emitted any scent, though doubtless they do so. A powerful odour which would attract the attention of an insect might, I suppose, be imperceptible to human olfactory organs. In any case, the bright golden hairs of the glands are plainly discernible many yards away as the insect is constantly thrusting the glands out and withdrawing them."

The only other Euploeine butterfly I have examined is **Tronga brookei,** Moore. This species has no brands or patches of special scales on the wing, and the brushes must therefore perform their scent-producing functions unaided. I have not thought it necessary to photograph any of the sections, as they could scarcely be distinguished from those of *T. mulciber*. The brush-bag possesses the same glandular

structure, though the hairs are far more irregular in transverse section. Whether owing to actual divergence in structure or to some difference in the condition of the specimen or method of preparation, the hairs in longitudinal view seem to show their structure more distinctly than in *T. mulciber*. Over a large part of their length they are covered with projections, the form of which I have endeavoured to show in Pl. XX, fig. 35. There is no dust-apparatus and no accessory vesicle attached to the brush-bag.

Hestia lynceus, Drury.

The species of the genus *Hestia* do not possess either scent-patch or pocket on the wings. They are, however, remarkable in having four abdominal brushes instead of two. Whilst the purpose of this modification is obscure, its origin is at least suggested by the condition of the brushes in such a species as *Amauris niavius*, where a separate tuft of stiff black hairs arises from a limited area of the brush-bag. The auxiliary brush in *Hestia* seems to represent a modification of this special tuft lying in a separate bag. It lies parallel with the main brush and rather above it. It is much shorter and more slender, the basal end being less deeply placed in the abdomen. It is of simple structure, contains but one kind of hair, and the containing bag appears to have a slightly glandular structure confined to one side. Pl. XVII, fig. 2, shows a section of one of these brushes beyond the glandular level of the bag. Near their origin the hairs are occasionally invaginated along one side, giving the section a reniform appearance. Under a high power the hairs present a close resemblance to those of the larger brush in the same species. Pl. XVII, fig. 3, shows a section of the large brush, close to the basal end. It will be observed that the brush-bag is formed of a mass of small cells, differing entirely in appearance from those found in the same situation in species of *Amauris*, but resembling the glandular cells in the brush-bag of *T. mulciber*. Pl. XVII, fig. 4, is a section taken rather further from the base, and shows much the same structure, which continues for some distance (fig. 5). Finally, the glandular structure disappears and the bag towards its mouth becomes a mere membrane (fig. 6).

The hairs of which the brush is composed, though well rounded at their origin, become somewhat flattened and

of irregular section towards their distal extremities. Some hairs contain particles of a brown secretion, which appears in the photographs as a blackening of the section. Pl. XX, fig. 38, is a section of one of the hairs at about the middle of its length, showing the contained granules, whilst fig. 39 taken nearer the distal end is without these. Fig. 37 is a surface view of one of the hairs somewhat beyond the middle of its length. The hair is covered with projections having a curious form, their outer surfaces bearing delicate ridges and their extremities produced to fine points. Pl. XX, fig. 36, shows the appearance of the glandular cells of the main brush-bag. It is scarcely possible to decide how many cells belong to each hair-socket. Possibly the number may vary. I have here shown three, since in the preparation from which the drawing was made three cells could be recognised with some certainty as attached to this particular socket. Beneath the entrance of the hair is a rod of protoplasm usually staining rather darker than the rest, and surrounded by vacuoles. The cells are very finely granulated and contain large and conspicuous nuclei. There is no dust material in *Hestia lynceus*. The absence of wing-glands and the glandular structure of the brush indicate that the scent-material is produced by the latter, being apparently secreted by the cells and discharged into the interior of the hairs. Thence it must make its way to the exterior, and doubtless openings exist, although I have not been able to discern them. It is possible that the passages, which must be very minute, may be hidden by the projections on the hairs.

Summary.

In the foregoing pages I have endeavoured to describe as concisely as possible the actual structure of the remarkable organs associated with the production of scents in a few species of tropical butterflies. It may be objected that in no case is there any direct evidence that a scent is produced at all. Such evidence cannot, however, be expected from examples which, for histological purposes, have been subjected to the chemical action of preservatives. It is furthermore to be admitted that Mr. Kershaw working in the field could detect no actual scent in *T. mulciber*. Nevertheless, we know from the observations of Fritz Müller, Dixey, Longstaff, and others that very many Lepidoptera do produce scents perceptible to the human

olfactory organs, and further that such odours can be definitely associated with wing-brands, tufts, scales, and other specialised portions of the anatomy of these insects. In the case of the Danaine brushes and brands we can have no doubt as to the function, or the association of that function with sexual purposes. Throughout the animal kingdom we are familiar with an infinite variety of elaborate devices providing for the continuance of the species, and in insects the olfactory sense seems pre-eminently chosen as the agent whereby the species may attract and find its mate. Perhaps the most remarkable feature of the organs here described is the elaborate development of friable hairs producing quantities of dust which almost certainly plays a very important part in the distribution of the scent, an interpretation strongly supported by the observed behaviour of *A. psyttalea*, and it is further remarkable that the dust may arise from either the brush or the wing according to the species. As I pointed out in my previous paper the dust material is no new discovery. The late Dr. Fritz Müller, whom little seems to have escaped, described such hairs in the wing-folds of Hesperidae and called them "chain-bristles," since they took the form of long hairs constricted at regular intervals and liable to break at each constriction. My friend Mr. A. H. Hamm has lately called my attention to large masses of yellow "fluff" occurring on the hind-wing of the male moth *Erebus macrops*. I find this to consist mainly of "chain-bristles" of an exceedingly beautiful form.

The studies described in the present paper show how a modest equipment of technique applied to suitable material may disclose much that is interesting in the mere structure of the organs in question, but of their physiology we know little or nothing. If the simple brush of *D. chrysippus* can perform its function efficiently what is the significance of the five different structures found in the complicated brush of *A. egialea*? The granules in the wing-cells of *D. lotis* are not of the same chemical nature as the secretion granules subsequently deposited in the brush. In what does the change consist? Do some of these brushes secrete a substance which combines with another stable compound provided by the wing patch to form a volatile oil? In *A. niavius* the wing-glands are neatly covered by a layer of scales which seem precisely adapted for the purpose of protection. Hence we might suppose that the

secretion of the wing is easily evaporated and must be protected, yet in *A. egialea* the scales cover the glands so imperfectly as to be probably useless for such a purpose.

It is to be feared that the study of the chemistry of these secretions is only possible in those countries where the insects may be obtained in large numbers and in fresh condition. Meanwhile I would proffer one or two suggestions to those collectors abroad who may take an interest in the subject. In detecting delicate odours and locating their origin it would seem that some success might be attained by using a glass tube one end of which would fit the human nostril the other being drawn out to a comparatively narrow diameter. The narrower end being applied to suspected centres of emission the odour might reach the human olfactory epithelium in a less diluted form than it necessarily does under ordinary circumstances. Furthermore, a number of crushed wing-brands might be placed in a small phial and warmed so that the scent might become sufficiently concentrated to be perceptible. Again, assuming a plentiful supply of material, might it not be possible to apply the principles of perfume manufacture to the extraction of any scent that may be present in certain organs? Large numbers of the scent-brands of some common species could be crushed and mascerated with a very small quantity of clarified lard and the product distilled with alcohol.* Or by means of a tube with a tightly fitting screw, and a minute aperture at one end, an appreciable quantity of the scent material might be expressed from a mass of the brands.

Finally, whilst fully recognising the excellent contributions already made to our knowledge of the subject by the authors mentioned in the course of this paper, it would seem that there still remain opportunities for comprehensive researches covering an interesting field, which it is to be hoped will attract the attention of our many capable workers in the tropics.

* Dr. Dixey has already shown that in the case of certain Pierines there may be made from the wings an alcoholic extract possessing the characteristic odour of the species from which it was prepared. See Proc. Ent. Soc. Lond., p. lix, 1905.

Explanation of Plates XI–XX.

[*See Explanations facing the* Plates.]

June 26, 1915