

ON HYBRIDS BETWEEN MOTHS OF THE GEOMETRID SUB-FAMILY *BISTONINAE*, WITH AN ACCOUNT OF THE BEHAVIOUR OF THE CHROMOSOMES IN GAMETOGENESIS IN *LYCIA* (*BISTON*) *HIRTARIA*, *ITHYSIA* (*NYSSIA*) *ZONARIA* AND IN THEIR HYBRIDS.

BY J. W. H. HARRISON, B.Sc., AND L. DONCASTER, Sc.D.

PART I. GENERAL ACCOUNT OF HYBRID *BISTONINAE*.

By J. W. H. HARRISON, B.Sc.

IN the *Entomologist* for July 1910 I indicated that I had succeeded in producing a number of new hybrids in the trio of genera included in what may be called, for the sake of convenience, the Biston group. I did not then, however, publish any details, either biological or otherwise, concerning them. Since then, in the case of many of these hybrids, I have worked out their detailed life history and have therein compared them, point by point, with the parent species. Further, I have secured several new and important pairings; not only is this so, but in the case of most of them I have published an exhaustive account in Oberthür's *Lepidopterologie comparée*, Fascicule VII. pages 333—655. In case this work is not readily accessible to English readers, I am writing this summary of my results to accompany Dr Doncaster's statement of his investigations into the oogenesis and spermatogenesis of *Lycia hirtaria* and *Ithysia zonaria*, and their two crosses, hybrids *denhami* and *harrisoni*. (Plate XVII.)

The hybrids reared may be divided into two sections.

- (a) The Primary hybrids, i.e. those with pure species as parents.
- (b) The Secondary hybrids, i.e. those in which either of the two parents was a primary hybrid.

The primary hybrids reared up to the present are as follows :

<i>Lycia hirtaria</i> ♂	× <i>Ithysia zonaria</i> ♀ (Pl. XVII)	= <i>Lycia</i> hybr. <i>denhami</i> .
<i>Lycia hirtaria</i> ♂	× <i>Ithysia graecaria</i> ♀	= <i>L. hybr.</i> <i>buloveci</i> .
<i>Lycia hirtaria</i> ♂	× <i>Poecilopsis pomonaria</i> ♀	= <i>L. hybr.</i> <i>pilzii</i> .
<i>Ithysia zonaria</i> ♂	× <i>Lycia hirtaria</i> ♀ (Pl. XVII)	= <i>L. hybr.</i> <i>harrisoni</i> .
<i>Ithysia zonaria</i> ♂	× <i>Poecilopsis lapponaria</i> ♀	= <i>I. hybr.</i> <i>merana</i> .
<i>Ithysia zonaria</i> ♂	× <i>Poecilopsis pomonaria</i> ♀	= <i>I. hybr.</i> <i>langei</i> .
<i>Poecilopsis pomonaria</i> ♂	× <i>Lycia hirtaria</i> ♀	= <i>P. hybr.</i> <i>hunii</i> .
<i>Poecilopsis pomonaria</i> ♂	× <i>Ithysia zonaria</i> ♀	= <i>P. hybr.</i> <i>helenae</i> .
<i>Poecilopsis lapponaria</i> ♂	× <i>Ithysia zonaria</i> ♀	= <i>P. hybr.</i> <i>smallmani</i> .

Similarly, the secondary hybrids are appended :

<i>Poecilopsis pomonaria</i> ♂	× <i>L. hybr.</i> <i>pilzii</i> ♀	= <i>P. hybr.</i> <i>brooksi</i> .
<i>Lycia</i> hybr. <i>pilzii</i> ♂	× <i>L. hirtaria</i> ♀	= <i>L. hybr.</i> <i>burrowsi</i> .
<i>P. hybr.</i> <i>hunii</i> ♂	× <i>L. hirtaria</i> ♀	= <i>P. hybr.</i> <i>hulli</i> .

Up to the present, only one tertiary hybrid, that is to say a hybrid with one of my secondary hybrids as a parent, has been obtained. This form has been successfully reared to the pupal state and I trust to have the pleasure of seeing the imagines next spring. Its parentage is *L. hybr. burrowsi* ♂ × *L. hirtaria* ♀ and I am calling it *L. hybr. adkinsi* in honour of my friend Mr. R. Adkin, who has assisted me in many ways during the course of my many experiments.

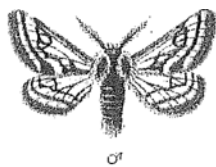
I have no intention of giving a prolonged account of the comments I made in my work in *Lepidopterologie comparée* upon the various phenomena observed which demanded special treatment. Two of these features, however (in my eyes at least), are so important that I have reserved them for a special paper to be published shortly.

The various points are given below and a brief *résumé* of the discussion in my longer paper is added to each.

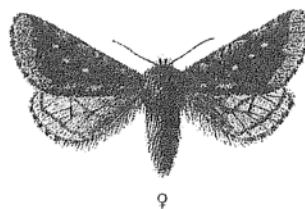
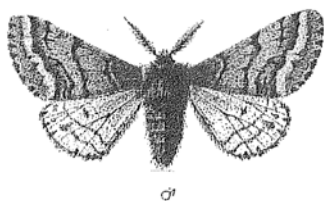
(1) *The great constitutional strength of the larvae.*

(2) *The growing sterility of the primary hybrids as the specific divergence between the parents increases.*

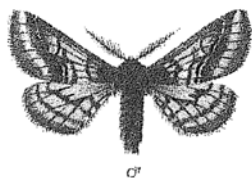
I pointed out that "strength" in the case of all of the crosses behaved as a Mendelian dominant, and that, granting the possibility of aberrations (mutations) possessing great constitutional strength, these two factors alone would result, in some cases, in what could only be classed as new species.



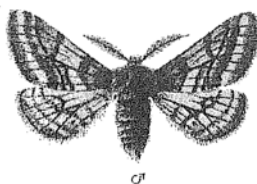
*Ithysia (Nyssia) zonaria.*



*Lycia (Biston) hirtaria.*



*L. hirtaria ♀ x I. zonaria ♂*



*I. zonaria ♀ x L. hirtaria ♂*

(3) *Dominance of characters derived from the undoubtedly weaker Ithysiae.*

This was treated at length and shown to depend on the fact that, highly specialised as the Ithysiae are, they are yet, in many points, the most primitive genus of the group in spite of the fact that, for the most part, the genus *Lycia* is nearer the common phylogenetic ancestor of both genera. In other words, we have a recurrence of common ancestral features.

(4) *The superior influence of the male.*

This is an experimental result that I have noted in practically all of the hybrids I have reared, not only in this family, but amongst the Ennomids and Larentiads likewise. No reasonable explanation of this fact has ever been suggested.

(5) *The refusal of the food plants of the Ithysiae.*

I have pointed out that this probably depends on the fact that, in general, the Boarmiads are an arboreal group and that the Ithysiae have almost certainly abandoned the family habit; there is thus latent in them a tendency to eat the same food plants as the *Poecilopsis-Lycia* fraternity.

(6) *The abnormal sex proportions yielded.*

Four of the hybrids,

*Lycia* hybr. *denhami*,

*L. hybr. buloveci*,

*P. hybr. smallmani*,

*P. hybr. helenae*,

exist only in the male sex under normal conditions.

It must not be supposed that this failure to give females is restricted to this compact group of genera, for I have observed it in the case of two other crossings I have made amongst the Boarminae, viz.:

(1) *Epione parallelaria* ♂ × *E. apiciaria* ♀ = hybr. *isabellae*

and (2) *Tephrosia crepuscularia* ♂ × *T. bistortata* ♀ = hybr. *bacoti*.

Strong inbreeding of the parents producing two of the above hybrids, on one occasion, resulted in the production of odd females, but the same procedure was without effect whenever *Lycia hirtaria* took part in the cross.

Five of the hybrids,

*L. hybr. pilzii*,  
*P. hybr. hunii*,  
*L. hybr. burrowsi*,  
*P. hybr. hulli*,  
*L. hybr. adkinsi*,

yield the two sexes in approximately equal numbers.

Three hybrids,

*P. hybr. langei*,  
*I. hybr. harrisoni*,  
*P. hybr. merana*,

give a large excess of females.

Lastly, the sole form reared from a hybrid ♀, i.e. *hybr. brooksi*, gives specimens which are hopelessly gynandromorphic, wings, body, genitalia, antennae being built up of parts chosen at random, as it were, from both sexes of the parents and grand-parents. It is worthy of note that the larger and more robust the specimen, the more the male characters predominate and *vice versa*.

(7) *The tendency for the hybrids to emerge long before the parent species.*

In most of the hybrids, it appeared that the emergence was hastened by a period varying from a fortnight to three months. Most curiously, this varied with the sex for, in most cases, the acceleration noted in the case of the females was very great compared with the slight displacement seen in the males, although the divergence was never so great as that observed in hybrid *robsoni* = *Larentia (Oporabia) dilutata* ♂ × *Lar. (O.) autumnata* ♀. In this case, the females appeared about four and a half months before the males and a similar period before both sexes of the reciprocal cross *rungei* = *L. (O.) autumnata* ♂ × *L. (O.) dilutata* ♀.

It is not my intention to give here my thoughts on the import of this, nor what appears to me to be the significance of the sex proportions detailed above. I hope to be in a position to publish my paper (already planned out) before long.

(8) *The wing development of the hybrid females.*

The hybrid females, when produced, vary greatly in their wing development; those between *hirtaria*, fully winged, of course, in both sexes, and one of the so-called apterous species, possess wings varying enormously even within the limits of the same brood. This is a distinct

consequence of the fact that, whilst there is but little difference in the wings of various specimens of *hirtaria* females, in the apterous forms there is an extraordinary amount of variability.

In *lapponaria*, for instance, the wings vary from mere vestiges to long lanceolate appendages and similarly with *pomonaria*; *zonaria* on the contrary, although not quite constant, for it varies slightly in the same direction, is almost so. The hybrids have thus to combine the immutability of the female wings of *hirtaria* with the varying forms developed in the other species. The result is that, whilst in most cases we have wings produced resembling very roughly ordinary male wings, although only two-thirds of the expanse, in many, we have long, narrow pointed wings, and in others, wings most curiously shortened, giving one the notion that a piece has been cut from them. As one might expect, the variation is least in the *zonaria* ♂—*hirtaria* ♀ cross and greatest in the two *hirtaria*—*pomonaria* crosses.

Nor is the variation confined to size and shape for, strange to say, the wing scales vary simultaneously; the smaller the wings the greater the tendency for the scales and fringes to become bristle or hair-like as in normal apterous females. Further, although the rudimentary wings of the apterous forms are not melanic, nevertheless the smaller the wings in the hybrids the greater the melanic tendency, which, however, it must be remarked, is present in all of the female hybrids.

In crosses between the apterous forms, in the majority of cases, the wings are small and constant, and seem greatly influenced by the wing form of *zonaria*.

The tendency of hybrid *brooksi* to produce forms with all the possible wing shapes combined in one specimen has already been noticed.

The wing forms of the other two secondary hybrids demand special attention. The females yielded by the crossing *humii* ♂ and *hirtaria* ♀ possess forewings about five-sixths of the normal wing expanse of *hirtaria* ♂ but with the inner margin shortened, and the costa and termen strongly rounded. The hindwings, except that they are proportionately stouter and broader, are not unlike those of *humii* ♀. The scaling of both sets is that of *hirtaria* ♀; the markings, too, except that the fringes are quite black, follow *hirtaria*.

The hybrid between *pilzii* ♂ and *hirtaria* ♀ very curiously produces females, which, except for the slightly rounded costa and termen, have both sets of wings almost, but not quite, as fully developed as those of *hirtaria* ♀. If the predominant influence of the male, as mentioned

above, is carried through both generations this would explain the difference in form between two hybrids so nearly the same in blood, both being three quarters *hirtaria* and one quarter *pomonaria*.

PART II. ON THE CHROMOSOMES IN GAMETOGENESIS  
OF THE MOTHS *LYCIA* (*BISTON*) *HIRTARIA* AND  
*ITHYSIA* (*NYSSIA*) *ZONARIA*, AND IN THEIR HYBRIDS.

By L. DONCASTER, S&D.,  
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The work of which this paper gives a preliminary account was undertaken to find out, if possible, any cytological cause for the fact that reciprocal crosses of *Lycia* (*Biston*) *hirtaria* and *Ithysia* (*Nyssia*) *zonaria* give different results as regards the sexes of the offspring. Mr J. W. H. Harrison, who very kindly supplied me with the hybrid material used, has described the results of the two crosses, together with other hybrids, between species of the same genera. For the present purpose the important facts are that the cross *zonaria* ♀ × *hirtaria* ♂ produces only male offspring, resembling *zonaria* somewhat more nearly than *hirtaria*; *hirtaria* ♀ × *zonaria* ♂ gives both sexes, with a conspicuous excess of females (more than 2 ♀♀ : 1 ♂). The males of this second cross are not strikingly different from those of the converse cross; the females are remarkable in having small flightless wings. (Plate XVII.) The wings of the *hirtaria* ♀ are of normal size and are capable of flight; those of *zonaria* are vestigial, so that in this respect the hybrid is intermediate<sup>1</sup>.

Although the observations which I have been able to make on the small supply of material hitherto available have not led to very satisfactory results with regard to cause of the difference in sex-determination in the reciprocal crosses, the general behaviour of the chromosomes in the gametogenesis of the hybrids is so remarkable that I think it worth while to publish an account of it at once. The chromosomes of the two species are very different from one another, and in the hybrids most of the chromosomes derived from each parent can be recognised at a glance.

<sup>1</sup> It should be noted that the reduction of the wings, which is a secondary sexual character of *zonaria*, is transmitted by the *zonaria* male.

Further, in the maturation divisions, as was found by Federley in hybrids of species of *Pygaera*<sup>1</sup>, most of the chromosomes are unpaired and divided singly, so that the spermatocyte divisions have nearly as many as the spermatogonia and oogonia, but my results differ from those of Federley in the fact that a few of the *hirtaria* chromosomes pair with some derived from the *zonaria* parent, and that there is a distinct synapsis stage in the prophases of the maturation divisions. The methods of preservation, etc., employed were the same as those described in my papers on gametogenesis of *Abraxas*, with the exception that the gonads were dissected out in water, which I have found, since those papers were written, to give better results than Ringer's fluid. The material of the pure species was obtained from quite young pupae purchased from Mr L. W. Newman; that of the hybrids, from larvae and pupae given me by Mr Harrison.

1. The chromosomes of *L. (B.) hirtaria*.

(a) *Oogenesis*. I have very few satisfactory figures of the oogonial divisions, but there seems to be little doubt that the chromosome number is 28. Plate XVIII, fig. 2 shows an oogonial equatorial plate, with 28 chromosomes varying considerably in size, and including six which are noticeably smaller than the remainder. I have one figure in which in addition to a similar group of 28, there is a body just outside the circle which might be a chromosome. I am inclined to believe that it is not a chromosome, but an extra-nuclear body (fig. 1).

A typical synapsis stage succeeds the last oogonial divisions, followed by a growth phase of the oocytes in which a relatively small number of fairly thick threads form a spireme. As the oocytes enlarge, these threads shorten in the usual way, and become faintly double, and by the time they have become elongated rods about thirteen can be counted (fig. 3). They then contract still further to double, or often faintly quadruple, bodies, of which again about thirteen may be counted. Meanwhile a chromatin-nucleolus has been conspicuous, and still remains much more sharply defined than the other chromosomes. It is compound, and in the many figures is seen to be composed of four parts, two small and two larger. The latter are frequently seen to be unequal in size. If the chromatin-nucleolus may be regarded, as in other forms, as a "sex-chromosome" there is thus perhaps an indication of its being unequally paired. There is no regularity, however, in its appearance; the parts may be together or widely separated, and are sometimes seen

<sup>1</sup> H. Federley, *Zeitschr. f. indukt. Abstamm. und Vererbungslehre*, ix. 1913, p. 1.



themselves to be composed of smaller units which show various degrees of separation. The counting of the double rods is not quite easy; usually thirteen seem to be present, but in some cases I cannot find more than twelve. If, as seems probable, there are thirteen, the larger and smaller members of the chromosome-nucleolus cannot consist of a large and small pair of united chromosomes, as occurs in the spermatogenesis (see below). Seiler has described the "sex-chromosome" in the female of the moth *Phragmatobia fuliginosa* as being double<sup>1</sup>, and the doubleness of the chromatin-nucleolus seen in *hirtaria* may perhaps be due to the same cause. As I have not investigated the polar divisions of the eggs, I cannot test the truth of this suggestion.

(b) *Spermatogenesis*. I have a number of clear figures of the spermatogonial divisions, and as in those of the oogonia there are clearly 28 chromosomes, of which six are smaller than the rest, and four of these conspicuously smaller (fig. 4). In the growth-stages of the spermatocytes a typical synapsis of the spireme occurs. As in other Lepidoptera, the stages are less easy to arrange in order than in the ovaries, but there is no doubt that a thin spireme thread, closely withdrawn to one side of the nucleus, is succeeded by a thicker and looser spireme (figs. 5, 6), and this by a reticulum, from which the double chromosomes of the first spermatocyte division arise by a process of concentration.

In the younger spermatocytes there are usually two chromatin-nucleoli; in the older ones commonly four, two larger and two small. These may be united so as to give three or two, a large one commonly being paired with a small one. I cannot find any constant difference in size between the two pairs; one of the larger or of the smaller is sometimes of greater size than its fellow, but this does not seem to be regular. The stages in which the chromatin threads contract into chromosomes are so confused that I am unable to count them, or in many cases even to distinguish the chromatin-nucleolus from the other chromosomes.

The primary spermatocyte divisions are usually extremely clear and diagrammatic, but are somewhat difficult to interpret, for in place of the 14 chromosomes which one would expect they constantly have only 13. Careful inspection, however, shows that one of the largest is compound, and consists of a large chromosome to which a very small one is attached (figs. 7, 8). That the large one is double (i.e. composed of an equally matched pair) is often quite clear, but the small one attached to it

<sup>1</sup> J. Seiler, *Zool. Anzeiger*, xli. 1913, p. 246.

usually shows no sign of doubleness, and on the spindle when seen sideways it sometimes appears to be attached to one half only of the large one, as if it were going entire to one pole of the spindle. This, however, appears to be exceptional; the compound chromosome is usually recognisable only with difficulty in side view (fig. 9, *a*, *b*, *c*). If it were the case that the small chromosome went undivided to one pole like a heterochromosome, the second spermatocyte equatorial plates should be of two kinds of equal frequency; half of them should have thirteen, and half either fourteen, or thirteen of which one should be compound. All these conditions in fact are found, but the great majority have thirteen of which one may be more or less clearly composed of a large and small member attached to each other with varying degrees of closeness. In some, perhaps the majority, only thirteen apparently simple chromosomes are visible; in others a large one clearly has a small one attached to it, and in a few fourteen separate chromosomes may be counted (figs. 11, 12, 13). In one of the latter class of figures, the largest chromosome has clear indications of being compound although there are thirteen separate chromosomes in addition to it, an arrangement which is undoubtedly abnormal (fig. 14).

Usually there are two chromosomes, in addition to the small member of the compound one, which are conspicuously smaller than the rest, but, probably according to the depth of staining and the position of the chromosomes on the spindle, there is some variation, so that only one, or sometimes three small ones, may be visible. It is thus not easy to identify any one chromosome with confidence, but when fourteen are visible, one very small one is usually, if not always, in close proximity to one of the largest. It is clear from this account that equatorial plates of the second spermatocyte divisions do not make it quite certain whether one chromosome goes over undivided in the first division or not. Unfortunately I have found only one case of a first division anaphase in which the chromosomes of both groups can be counted (fig. 10), and this is not entirely unequivocal. In each group there are thirteen chromosomes, one of which shows signs of being composed of a larger and smaller unit in each group. This would indicate that the double chromosome divides equally, a large and a small portion going to each pole. In one group, however, there is a body outside the group, and at a different level in the section, which might possibly be a chromosome. It is nearer the pole than the group of thirteen, and I am fairly confident that it is not a chromosome but an extra-nuclear body. Such occur regularly in the spermatocyte cells.

Although, therefore, there are some appearances which suggest the presence of a heterotropic chromosome in the male, I am inclined to believe that these are deceptive, and that one of the smallest chromosome pairs constantly unites with one of the largest in the first spermatocyte division, and divides normally, but that the closeness of the union varies in different cases in the second division, so that sometimes thirteen, sometimes fourteen, appear to be present.

2. The chromosomes of *Nyssia (Ithysia) zonaria*.

(a) *Spermatogenesis*. A glance at a spermatogonial equatorial plate shows at once that the chromosomes are very unlike those of *L. (B.) hirtaria*. Instead of 28 rather large chromosomes, there are a very large number of extremely small ones. I have found no figure in which they can be counted with accuracy; they tend to come into contact and one can only make out with confidence that there are over 100 (fig. 15).

The synapsis and growth phases are closely similar to those of *hirtaria*, except that the "bouquet" stage (fig. 16) is less typical; as in that species there are sometimes four chromatin-nucleoli, consisting of a larger and smaller pair, but more frequently these are united to form three or two. They are very nearly of the same size as those of *hirtaria*. The prophases of the first spermatocyte division are clearer than in *hirtaria* owing to the much smaller size of the chromosomes, and it can be seen that the chromatin-nucleoli approach one another more and more closely, and finally unite into a single rounded mass in which no division can be seen.

The primary spermatocyte divisions show equatorial plates of really surprising beauty and perfection (fig. 17). The chromosomes lie absolutely in one plane, and widely separated from one another, so that there is not the smallest difficulty in counting 56 with complete certainty. Of these, two are noticeably larger than the rest, and usually two others intermediate in size. The number 56 is so clearly and certainly shown, that one may conclude with confidence that the spermatogonial (diploid) number is 112, that is to say, four times that of *hirtaria* (28). The largest chromosomes of *zonaria* are of about the same size as, or possibly even smaller than, the smallest of *hirtaria*. In metaphase and anaphase it is seen that each chromosome is dividing in the normal "heterotype" manner, the diverging halves being connected by double strands, and I can find no evidence of unequal division, nor of the union of two pairs, such as occurs in *hirtaria*.

The second spermatocyte division resembles the first very closely, except that the chromosomes are half the size; 56 can easily be counted, of which four are larger than the others (fig. 18).

Some follicles of both *zonaria* and *hirtaria* have abnormal spermatocyte divisions, leading to spermatozoa without nuclei, as in *Pygaera*, *Abraaxas*, etc.

(b) *Oogenesis*. My *zonaria* pupae were too old to give satisfactory observations on the oogenesis. The ovaries were already large, with eggs at the lower end of the tubes in which a considerable amount of yolk had been deposited, so that the tubes were becoming moniliform. I have found no diploid mitotic figures in which the chromosomes can be counted accurately. The younger oocytes at the top of the tube had already undergone synapsis, and were in the stage with the chromosomes arranged under the nuclear membrane. Completely accurate counts of this stage are scarcely possible when the chromosomes are numerous; it can only be said that there are between 50 and 60 small double chromosomes, and a composite chromatin-nucleolus of which the two largest portions are almost always of recognisably unequal size.

### 3. Chromosomes of the Hybrids.

(a) *Zonaria* ♀ × *hirtaria* ♂. This cross gives only male offspring. My material consists of testes of two larvae shortly before pupation, and of one pupa about three weeks old. The larval testes contain no divisions later than the spermatogonia; the pupal testis has also first and second spermatocyte divisions, and contains spermatids in an advanced stage of development towards spermatozoa.

The spermatogonial equatorial plates show at a glance two kinds of chromosomes—comparatively few large ones intermingled with a much larger number of small ones.

A count of a very good figure (fig. 19) gives 55—57 small and 14 large; although this cannot be regarded as absolutely accurate, the error certainly does not amount to more than two or three small chromosomes at most. The theoretical expectation is 70, so that it may be assumed that complete haploid sets of *hirtaria* and *zonaria* chromosomes are present.

The spermatogonial divisions are succeeded by a stage in which a thin spireme is contracted to one side of the nucleus, after which the thread thickens somewhat and becomes reticular, but the typical "bouquet" stage (pachynema), which is found in pure *hirtaria* and less

typically in *zonaria*, seems not to occur (figs. 20, 21). In the mature spermatocyte there are either one or two chromatin-nucleoli which are clearly compound; the parts show less tendency to become separate than in either of the pure species.

Federley, in his work on hybrids between species of *Pygaera*, found no synapsis (synizesis) stage in the hybrids; in the present case, although most of the chromosomes fail to pair, so that there is almost the diploid number in the spermatocyte divisions, there is no important difference between the spermatocytes in the earliest growth stage of the hybrids and those of the parent species. The fact, however, that I have found no "bouquet stage" with thick thread still contracted to one side of the nucleus, is probably to be correlated with the fact that most of the chromosomes fail to pair. Federley's failure to find any synapsis (synizesis) in *Pygaera* hybrids may possibly be due to his material being too old. In the present case I find such a stage very frequently in larval testes, but not in pupal testes about a month older, in which the majority of the follicles contain advanced spermatocytes, and the earlier stages are scarce. The various stages seem to overlap less in the hybrids than in the pure species.

The spermatocyte division figures are very remarkable, and are not conspicuously different from those of the spermatogonia. I have a number of very perfect figures, and in all it is quite clear that the chromosomes are nearly in the somatic number (figs. 22, 23), as was found by Federley in his hybrids with *Pygaera* spp. Careful counts show, however, that the full somatic number is not present, and that some pairing of chromosomes has taken place. It is not easy to draw the line quite clearly between the large and small chromosomes, for as was said above the larger *zonaria* are similar in size to some of the smaller of *hirtaria*. In the first spermatocyte equatorial plate there are always about 12 or 13 which are certainly larger than all the rest, and most of these may be regarded as *hirtaria* chromosomes. The number of small ones is commonly about 50; careful counts have given 50, 50 or 51, and 51 or 52, in the three best figures I can find. Another fair figure (slightly oblique, fig. 23) in which I cannot find that any chromosomes are omitted from the section, gives 13 large and about 40 small, and others have given intermediate numbers; it is possible that some are covered by others, but it is unlikely that so many would disappear in this way, and probably the smaller number may be due to the fact that more chromosomes find mates in some cases than in others. There seems no doubt that the total does not amount to the theoretical number

of 69 or 70 ( $56 + 13$  or  $14$ ) which would be expected if no pairing of chromosomes took place at all. This is further confirmed by the fact that there are constantly 12—14 large ones. *Hirtaria* spermatocytes have eleven large and two small, and the largest of *zonaria* are hardly big enough to be classed in the "large" group. If, however, the larger of the *zonaria* chromosomes paired with the smaller of the *hirtaria* chromosomes, these would make additional large ones in the hybrid spermatocyte equatorial plate. Several, in fact, often appear to be double, and as the number in the best figures adds up to 65, it may be concluded that about five *zonaria* chromosomes (probably the largest) pair with five derived from *hirtaria*, while most of the rest remain unpaired. An examination of the prophase figures of the first spermatocytes just before the nuclear membrane disappears, shows, in addition to several clumps of three or more chromosomes together, a large number of single chromosomes of various sizes, and among them a few which are paired, either equally or unequally (fig. 24). Further confirmation of the evidence that some of the chromosomes are paired in the first spermatocyte division and that the smaller number counted is not due to error, is found in the fact that almost exactly the full number can be counted in the spermatogonial divisions, which are smaller and less easy to examine accurately than those of the spermatocytes. If it is easy to count very nearly 70 in the spermatogonia, it is hardly possible that the smaller number in the spermatocytes can be due to error.

In the first spermatocyte division, it appears that not all the chromosomes divide. Most undoubtedly do, but some show no signs of division in metaphase when seen from the side of the spindle (fig. 25), and the appearance of the secondary spermatocyte equatorial plates confirms this supposition. The chromosomes in these second division figures are less easy to count with complete accuracy, but it is not difficult to get a fairly close estimate of their number, and this is almost constantly less than in the first division (fig. 26). Counts have given 9 large and 42 small, 9 large and 46 small, 12 large and 42 small. In one case, where I counted 12 large and 52 small, it is almost certain that several had already divided, and that the halves were counted as separate chromosomes.

(b) *Hirtaria* ♀ × *zonaria* ♂. This cross gives a preponderance of females, with some males. The ovaries of full-grown larvae are extremely small and difficult to find, and from the three female larvae sent me by Mr Harrison I only succeeded in getting one ovary.

I allowed one larva to pupate; it was fortunately a male and the testis was preserved about two weeks after pupation.

The single larval ovary is most unfortunately not very well preserved. It contains only one oogonial equatorial plate which is sufficiently in face for the chromosomes to be seen at all clearly, and it is not good enough to provide an accurate count of the chromosomes. It shows, however, a mixture of large and small chromosomes such as I have described in the spermatogonial plates of the converse cross. Other oogonial divisions, which lie more obliquely, also show chromosomes of two very different sizes. Owing to the defective preservation, the development of the oocytes cannot be made out completely. There is a stage with a thin spireme thread massed at one side as in the normal early synapsis stage, and lower down the tube there are larger nuclei with a thicker thread, but I have not found any nuclei in which the thread has segmented into separate chromosomes, such as are found in normal older oocytes. The ovary, however, is so small that this might perhaps not have occurred at this stage of development even if normal synapsis had taken place. Since the development of the chromosomes is much more clearly seen in oocytes than in spermatocytes of Lepidoptera, it is disappointing that in my only ovary the stages required are not represented.

*Spermatogenesis.* The testis of the single male pupa is large and well developed, and contains all stages up to the spermatids, but no nearly mature spermatozoa such as would probably occur in either of the parent species at the same date. The spermatogonial divisions are like those of the converse cross; I have not been able to make counts which I can regard as completely accurate, but in the equatorial plate from which fig. 27 is taken the chromosomes are clear enough to give some approach to accuracy. I count in this plate 14 large and 55 small; in other plates I have counted only 11 or 12 large, and it is probable that two or three reckoned as large in the group figured are *zonaria* rather than *hirtaria* chromosomes. It is also possible that two or three small ones counted as two are really single ones in division. The number is sufficiently near to the theoretical expectation of 70 to make it almost certain that complete sets from each parent are present.

The early stages of the development of the spermatocytes show quite clearly cells with a fine spireme contracted to one side of the nucleus; it does not differ conspicuously from the corresponding "contraction-phase" of synapsis in the pure species (fig. 28). After this stage the nuclei enlarge considerably, and the thread takes the

form of a reticulum, as in the converse cross (fig. 29). There are usually at this stage two chromatin-nucleoli, each of which is double, with halves which are slightly unequal; the parts of one or both may become separate, so as to produce three or four distinct masses.

The testis includes good equatorial plates of both first and second spermatocyte divisions. Counts of first division figures have given numbers ranging from 52 to 60 chromosomes (figs. 30, 31); in those with the higher numbers it is possible that one or two dividing pairs have been counted as two singles in each case. There seems no doubt that the total number is not more than 60, and usually several less, and as in some figures as many as 18 have been reckoned as large, it is practically certain that a considerable amount of pairing has taken place, since only about eleven large ones are introduced from the *hirtaria* parent, and the remaining large ones must be formed of pairs of small ones. This is confirmed by the second spermatocyte divisions, in which only about 10 large ones are counted; the small chromosomes in this division are so small as to be counted with difficulty, but the total of second equatorial plates is clearly not far from 50, made up of about 10 large and 40 small (fig. 32). It is probable, therefore, that in the cross *hirtaria* ♀ × *zonaria* ♂ more chromosomes find mates in the maturation divisions than in the converse cross, in which over 60 are usually present in the first spermatocyte divisions, but as a lower number was found in some equatorial plates of this cross, it is probable that the number which pair varies somewhat in different cells.

#### *Conclusions and Discussion.*

The general conclusions which may be drawn from the observations described are then as follows: (1) there are 28 somatic chromosomes in *hirtaria*, giving 13, of which one consists of a large united to a small, in the spermatocytes. (2) There are 112 somatic and 56 spermatocyte chromosomes in *zonaria* which are much smaller than most of those of *hirtaria*. (3) The spermatogonial number of the hybrids, whichever way the cross is made, is as nearly as can be counted 70, of which fourteen are conspicuously larger, and of these twelve are derived from the *hirtaria* parent, the other two from *zonaria*. (4) The early synapsis stage of the spireme in the young spermatocytes and oocytes of the hybrids does not differ greatly from that of the pure species, but it is apparently not followed by a normal pachytene ("bouquet") stage with thick coiled thread. (5) Comparatively few of the chromosomes are



paired in the spermatocyte divisions, so that these have only from 5 to 15 fewer than the somatic number. It is quite clear, however, that some chromosomes, the number of which is small and probably somewhat variable, do find mates in the maturation divisions, and that there are probably homologous chromosomes in the two species which pair together, while the remainder are unable to do so.

I do not propose to discuss at length the observations of which only a condensed account has been given, firstly because to a considerable extent they are similar to those of Federley, and he has discussed the questions raised at considerable length; and secondly because I hope next year to complete the investigation on additional material of the same and other species, which should make a fuller understanding of the problems possible. There are, however, a few points which seem to require comment.

The chief points of interest are (1) the difference in chromosome number in nearly related species, and the behaviour of the chromosomes in the hybrids, and (2) the possible causes of the fact that the cross *zonaria* ♀ × *hirtaria* ♂ gives only male offspring, while the converse cross gives both sexes with excess of females.

In the former group of phenomena we have important data on the vexed question of the individuality of the chromosomes. Since there are exactly four times as many chromosomes in *zonaria* as in *hirtaria*, and since the *zonaria* chromosomes are so much smaller that the total amount of chromatin, as judged by the size of the resting nuclei, is approximately equal in the two species, it seems a fair inference to suppose that the *hirtaria* chromosomes may be regarded as compound, made up of units corresponding with the separate chromosomes of *zonaria*. Since however there are large and small chromosomes in *hirtaria*, and some in *zonaria* which are noticeably larger than the rest, it is probable that not every *hirtaria* chromosome is made up of four parts each of which corresponds with one *zonaria* chromosome, but that the larger *hirtaria* chromosomes are made up of more than four units, and the smallest ones are probably single. That is to say, if there are 56 units in the haploid group of *zonaria*, there are the same number of units in *hirtaria*, but these are combined with one another in various ways so that only 14 (or 13) chromosomes appear. For example, if the 56 haploid *zonaria* chromosomes are taken as units, the haploid set of *hirtaria* might be made up of four chromosomes each composed of six units, five of five, one of four, and three of one unit each. The total 56 is thus made up ( $4 \times 6 + 5 \times 5 + 1 \times 4 + 3 \times 1$ ). This is of course not

the only possible arrangement; I give it merely as an illustration of the way in which I regard the *hirtaria* chromosomes as perhaps being composed of a variable number of units each corresponding with one *zonaria* chromosome. It is of course probable, if the *hirtaria* chromosomes are compound, that those of *zonaria* are so also, and that the true "unit," whatever that may be, is smaller than either. Meek<sup>1</sup> has shown that the chromosome lengths in various species of insects and other animals are terms of an arithmetical series, as if they were composed of units of standard length, and the comparison of *hirtaria* with *zonaria* leads by a different route to a somewhat similar conclusion.

Another aspect of the individuality question is touched by the phenomena of the maturation divisions of the hybrids. I have shown that although the majority of the chromosomes fail to pair in synapsis, it is certain that some of them do so. The number of those which pair is sometimes not more than ten (five pairs), giving a maximum number of 65 in first spermatocyte equatorial plates in the cross *zonaria* ♀ × *hirtaria* ♂; in other cases, especially in the converse cross, the number of pairs appears sometimes at least to be larger. If I am right in suggesting that about three of the small *hirtaria* chromosomes correspond with single *zonaria* units, we might expect these to pair normally together, and the indications of occasional unequal pairing suggest that some of the compound *hirtaria* chromosomes may pair also, perhaps less regularly, with *zonaria* units. This would lead to the conclusion, not that chromosomes are individuals in the sense of being indivisible units, but that they are composed of units, and that pairing in synapsis is due to some affinity between chromosomes made up of similar components. This is of course the hypothesis which has been widely accepted on evidence of a different kind, in connexion with the hypothesis that the chromosomes are the bearers of Mendelian unit characters.

If there is any truth in this view, it may give some clue to the baffling problem of interspecific sterility. The *hirtaria*—*zonaria* hybrids are completely sterile. If the haploid set of 56 chromosomes of *zonaria* consists on the whole of the same units as the 14 haploid chromosomes of *hirtaria*, combined in different ways, the sterility between the two nearly allied species may have arisen simply from a difference of grouping. Since the units are grouped differently, the chromosomes cannot pair properly in synapsis, and this may be the cause of the

<sup>1</sup> C. F. U. Meek, "A metrical analysis of chromosome complexes, etc.," *Phil. Trans. Roy. Soc.* Vol. B. 203, p. 1, 1912.

sterility. It is true that Federley's *Pygaera* hybrids were not sterile, but that may be because little or no pairing took place, so that the resulting gametes would be less abnormal than in a case where some chromosomes pair and others do not.

The second point mentioned at the beginning of this discussion was the one which led in the first instance to my undertaking the work—the possible cause of the fact that only males are produced from the cross *zonaria* ♀ × *hirtaria* ♂. Similar results have been obtained with other species by various observers, notably the cross *Tephrosia bistortata* ♀ × *T. crepuscularia* ♂ described by Tutt<sup>1</sup>. Brake and Goldschmidt's observations on *Lymantria dispar* and *L. japonica* may also be compared, in which they find that *dispar* ♀ × *japonica* ♂ gives males and gynandromorphs, although the converse cross gives normal males and females<sup>2</sup>. As Mr Harrison has shown, true hermaphrodites occur in certain crosses with the genus *Biston*, but not with those used in the present case.

My observations on the chromosomes do not yet give any conclusive results with regard to the cause of these sex-phenomena, but there are certain indications which perhaps deserve mention. It is probable that in Lepidoptera there are two similar "sex-chromosomes" in the male, and that one of these differs from the other, and may be regarded as lacking the male determiner, in the female. This has been suggested on the ground of the facts of sex-limited inheritance (*Abraxas*); the suggestion is supported, though not proved, by the observations of Seiler, of which only a preliminary account has been published<sup>3</sup>; and I have obtained additional evidence for it from a study of the chromosomes in a strain of *Abraxas* which in each generation produces families consisting only of females. In a first account of this work<sup>4</sup> I have shown that females of this strain have 55 instead of 56 chromosomes, and further work, an account of which I hope will be published shortly, confirms this observation, and shows that all spermatozoa have 28 chromosomes, while eggs have either 28 or 27.

In *B. hirtaria*, although there is no certain indication as to which chromosome is the "sex-chromosome," I think it may be assumed with some probability that it is one of the larger ones. One of the largest is always coupled with a small one in the first spermatocytes and such

<sup>1</sup> J. W. Tutt, *Trans. Entom. Soc.* 1898, p. 17.

<sup>2</sup> R. Goldschmidt, *Zeitschr. f. indukt. Abstamm.* VII. 1912, p. 1.

<sup>3</sup> L. Seiler, *Zool. Anz.* XLI. 1913, p. 246.

<sup>4</sup> *Journ. of Genetics*, III. 1913, p. 1.

coupling has frequently been described in connexion with heterochromosomes or "sex-chromosomes"; the size of the chromatin-nucleolus also indicates that it would give rise to one of the larger chromosomes. Now all the larger chromosomes of *hirtaria* are of greater size than any of *zonaria*, so that if the size of the sex-chromosome is any indication of its intensity of action, it is probable that in the cross *zonaria* ♀ × *hirtaria* ♂, in which all the spermatozoa have the large male-determining chromosome of *hirtaria*, all the zygotes might receive a sufficient quantity of the male-determining substance to cause them to become male, whether they received any from the female parent or not. In the converse cross, only half the eggs would receive a male sex-determiner from the *hirtaria* ♀, and therefore both males and females would be produced.

This suggestion does not differ essentially from that used by Goldschmidt to explain the results obtained with *Lymantria dispar* and *L. japonica*, the essence of which is that the "potency" of the sex-determiner may be different in different species. It is founded, however, on observations on the size of the chromosomes rather than on the simple results of breeding experiments. It is admittedly very hypothetical, and does not explain the excess of females which seems constantly to occur in the cross *hirtaria* ♀ × *zonaria* ♂. I hope to be able next season not only to amplify my work on the species used this year, but also to examine other crosses which give comparable results, and to confirm or disprove the hypothesis suggested by observations on ampler and more varied material.

## EXPLANATION OF FIGURES.

### PLATE XVIII.

All the figures were drawn free-hand, with a Zeiss 3 mm. 1.40 ap. immersion objective and Compens. oc. 12.

[N.B. In Figs. 19, 22, 23, 26, 27, 30, 31, 32 the reproduction has made some small chromosomes appear in contact which in the original were near together but quite distinctly separate.]

Figs. 1—14. *B. hirtaria*.

Figs. 1, 2. *B. hirtaria* oogonial equatorial plates. 28 chromosomes in each, if the small body at the top of Fig. 1 is not a chromosome. There are in each four very small and two rather small chromosomes.

Fig. 3. Oocyte nucleus, cut in two sections. Three chromosomes are cut in both sections (lettered *a*, *b*, *c* in each). Thirteen elongated chromosomes showing traces of doubleness, and compound chromatin-nucleolus (*ch.n.*).

Fig. 4. Spermatogonial equatorial plate. Four very small and two rather small chromosomes. Total 28.

- Fig. 5. Early synopsis stage; fine thread withdrawn to one side of nucleus.
- Fig. 6. Later synopsis (bouquet or pachytene stage), with coiled thick thread.
- Figs. 7, 8. Primary spermatocyte equatorial plates. 13 chromosomes, of which one consists of a large coupled with a small (left upper side in Fig. 7, right lower in Fig. 8).
- Fig. 9, *a, b, c*. The compound chromosome in side view. In *b* the small member appears not to be dividing; more commonly in metaphase all the large chromosomes appear like *c*.
- Fig. 10. Primary spermatocyte anaphase; two daughter groups, cut in successive sections. In the left group there are 13, one of which is compound; in the right group there are 13 with a stained body outside the circle which is probably not a chromosome.
- Figs. 11—14. Secondary spermatocyte equatorial plates. In Fig. 11 there are 13, one of which is clearly compound; in Fig. 12, none is obviously compound; in Fig. 13 the small member is quite separate from the large; Fig. 14 an abnormal figure in which there are 14, one of which is compound.
- Figs. 15, 16, 17, 18. *B. zonaria*.
- Fig. 15. Spermatogonial equatorial plate. The chromosomes are so small and crowded that an exact count is impossible. There are more than 100 and less than 120.
- Fig. 16. Pachytene stage of spermatocyte. The thread is thinner than in *hirtaria*, and takes a less typical "bouquet" form.
- Fig. 17. Primary spermatocyte equatorial plate. 56 chromosomes quite clearly, of which four are larger than the rest.
- Fig. 18. Secondary spermatocyte equatorial plate. 56 chromosomes, of which four are larger.
- Figs. 19—26. Hybrid, *zonaria* ♀ × *hirtaria* ♂. (This cross yields only males.)
- Fig. 19. Spermatogonial equatorial plate. 14 large chromosomes and about 56 small.
- Fig. 20. Early synopsis.
- Fig. 21. Thread becoming reticular, apparently omitting the bouquet stage.
- Figs. 22, 23. Primary spermatocyte equatorial plates. In Fig. 22, 12 or 13 large and 50 or 51 small; in Fig. 23, 13 large and about 40 small; several of the latter are of intermediate size, as if consisting of pairs.
- Fig. 24. Typical chromosomes from primary spermatocyte prophase, showing single, equally paired, unequally paired and grouped chromosomes.
- Fig. 25. Outline of part of primary spermatocyte spindle in side view, showing some chromosomes dividing, others apparently not.
- Fig. 26. Secondary spermatocyte equatorial plate—about 9 large and 46 small.
- Figs. 27—32. Hybrid, *hirtaria* ♀ × *zonaria* ♂. (This cross gives females and males.)
- Fig. 27. Spermatogonial equatorial plate. About 14 large and 55 small chromosomes.
- Figs. 28, 29. Early and later synopsis stages, corresponding to Figs. 20 and 21 of the converse cross.
- Figs. 30, 31. Primary spermatocyte equatorial plates. In Fig. 30 about 11 large, 8 or 9 intermediate, and about 40 small; in Fig. 31, 11 large and 42 intermediate and small.
- Fig. 32. Secondary spermatocyte equatorial plate. About 9 large and 41 small.

