THE PARENTS AND PROGENY OF *AESCULUS CARNEA*

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(With Plates I and II and Twenty-four Text-figures)

Introduction

Aesculus carnea arose more than a century ago presumably as a chance hybrid between *Ae. Hippocastanum*, the European horse chestnut, and *Ae. Pavia*, the North American buckeye. *Ae. Hippocastanum* grows to a height of 80–100 ft., has resinous buds, white flowers and prickly fruits. Its five or seven large leaflets are sessile upon the petiole. *Ae. Pavia*, once placed in a separate genus *Pavia*, is a shrub never taller than 18 ft. with non-resinous buds, red flowers and smooth fruits. It has five leaflets, all of which are stalked. The characters of the hybrid are intermediate between those of the two parents (Plate I *a*). *Ae. carnea* is a tree of about 30–40 ft., its buds slightly resinous, its flowers pink and its fruits somewhat spiny. The leaflets are intermediate in size between those of *Ae. Hippocastanum* and *Ae. Pavia* and are attached to the petiole by very short stalks. Although it is somewhat less fertile than *Ae. Hippocastanum*, its seeds germinate readily and it breeds true (Bean, 1914).

The explanation of this unexpected behaviour lies in the fact, discovered by Hoar (1927), that *Ae. carnea* is tetraploid, having 80 chromosomes, while all the other species of the genus have 40. This account was confirmed by Skovsted (1929). He points out that the new species was derived presumably by the doubling of a sterile hybrid, as in the classical case of *Primula kewensis*. But he goes further. He states that the complement of *Aesculus carnea* is made up of 40 large chromosomes derived from *Ae. Pavia* and 40 small ones from *Ae. Hippocastanum* which can be distinguished from one another at the first metaphase of meiosis. A similar case has been described by the same author in cotton (Skovsted, 1935).

There are many examples of chromosomes of different sizes derived from different parents pairing at meiosis. In these cases the partners differ structurally, that is, one has gained or lost segments by structural

change in the course of its history. But where two species, like the parents of *Ae. carnea*, differ, as Skovsted states, uniformly in size, no such explanation is possible. The one cannot have uniformly lost part of each chromosome, nor can the other have uniformly gained. Such a difference must be genotypically controlled unless it is determined by some special "accessory substance" in the chromosomes themselves (Darlington, 1932 *a*). Many cases of hybrids and mutants showing this genotypic control of size are known. For example, Navashin (1931) describes a hybrid between *Crepis capillaris* and *C. neglecta* in which the chromosomes derived from *C. capillaris* are longer and those from *C. neglecta* shorter than they are in the parental species. Similarly a triploid *Tradescantia*, presumably a hybrid between the tetraploid *T. virginiana* with large chromosomes and a diploid species with smaller ones, had chromosomes as large as those of the tetraploid, with the exception of one bud, in which the chromosomes and the nuclei were reduced by mutation to one-fifth of their normal size (Darlington, 1929 *b*). It is an essential property of these hybrids that genotypically controlled differences between the chromosomes of the parents disappear in the hybrid when they are brought under a new and uniform genotypic control.

Aesculus carnea is therefore, according to Skovsted's description, an exception to a general rule, and as such calls for specially careful examination. For this reason I undertook to reinvestigate it, together with its parents and its sterile derivative *Ae. plantierensis*.

Material and methods

The anthers are small at the time of meiosis, and the pollen mother cells stick together somewhat; yet I found that smearing was the most satisfactory method of obtaining preparations. In *Ae. Pavia* the pollen mother cells are more difficult to smear than in the other species, and the division very rapid, so that very few metaphases could be obtained. In this species therefore, in addition to making smears, I dissected out the anthers and fixed them for embedding.

Root tips were obtained by taking the lateral roots from freshly germinated chestnuts. The main root is useless on account of the accumulated starch and its great size, which prevents rapid penetration of the fixative. Anthers were cut at 10 μ and root tips at 6 μ.

All preparations were fixed in 2 BE (La Cour, 1931) and stained in Newton's gentian violet. In staining the pollen mother cells of *Ae. Pavia* and the root tips of all species, it was necessary to leave the slides in the

gentian violet for half an hour and mordant in iodine for about 10 min. before passing rapidly through the alcohols into clove oil.

The material examined came from the following sources:

Species	Root tips	Pollen mother cells
Ae. flava $2n=40$	Garden of Sir George Cooper, Winchester	Royal Botanic Gardens, Kew
Ae. Pavia $2n=40$ (1)*	Botanic Garden, Copenhagen	—
(2)	Royal Gardens, Windsor	—
(3)	—	Royal Botanic Gardens, Kew
Ae. Hippocastanum $2n=40$	John Innes Hort. Inst., Merton	John Innes Hort. Inst., Merton
Ae. carnea $2n=80$	John Innes Hort. Inst., Merton	John Innes Hort. Inst., Merton
Ae. plantierensis $2n=60$	—	Royal Botanic Gardens, Kew

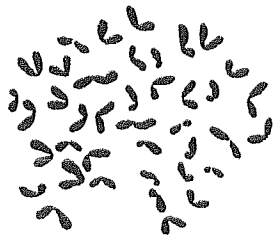
* There appears to be some doubt as to the identity of this plant. It is probably *Ae. flava.*

Ae. plantierensis is a sterile species presumed to be a back-cross between *Ae. carnea* and *Ae. Hippocastanum* which was raised in the nurseries of Messrs Simon-Louis Frères at Plantières near Metz (Bean, 1914).

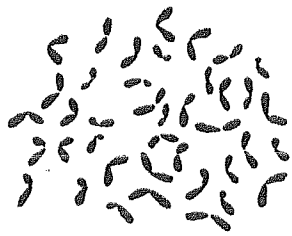
MITOSIS AND MEIOSIS

The genus *Aesculus* is divided into two groups on morphological grounds, the Eu-Aesculus section and the Pavia section. The former, to which *Ae. Hippocastanum* belongs, has, according to Skovsted, chromosomes about one-eighth the size of those of the Pavia section, of which *Ae. Pavia* and *Ae. flava* are members. I find no such difference either in the somatic divisions (Text-figs. 1–5) or at meiosis (Text-figs. 6–11). There are, however, slight differences in size within each complement, and these are comparable. The largest chromosomes have median centric constrictions and are about 1·3–1·5 μ in length. The smallest have subterminal constrictions and are about 0·5–0·6 μ. Measurements of this order are necessarily only of comparative value, since the wave-length of green light used for observation is itself 0·5 μ. Precisely similar differences occur within the complement of *Ae. carnea* (Text-fig. 5). Furthermore in spite of their small size it was possible to pick out two chromosomes with trabants, two with very long centric constrictions and two with secondary constrictions. These constrictions are probably associated with the formation of the nucleoli (cf. Text-figs. 6, 7). Probably such chromosomes also occur in one or other of the parental species, but the preparations were not sufficiently critical to show such fine details.

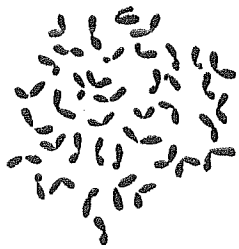
Metaphases flat enough to photograph could be found only in *Ae. Hippocastanum* and *Ae. Pavia* (Plate I *b*).



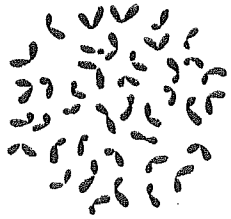
Text-fig. 1. *Ae. flava* $2n=40$



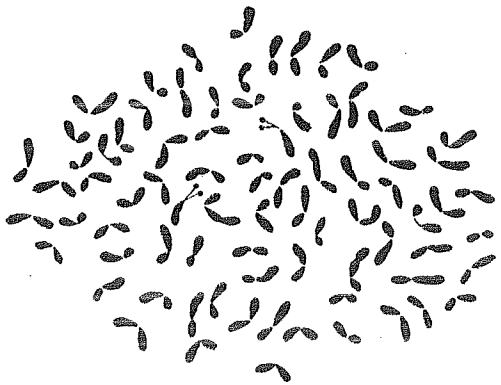
Text-fig. 2. *Ae. Hippocastanum* $2n=40$



Text-fig. 3. *Ae. Pavia* $2n=40$ (from Windsor)



Text-fig. 4. *Ae. Pavia* $2n=40$ (from Copenhagen)



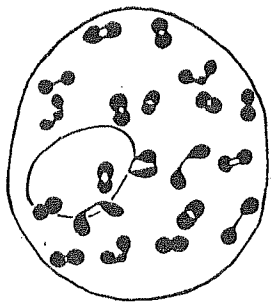
Text-fig. 5. *Ae. carnea* $2n=80$

Text-figs. 1–5. Somatic divisions from the root tip. ×4800.

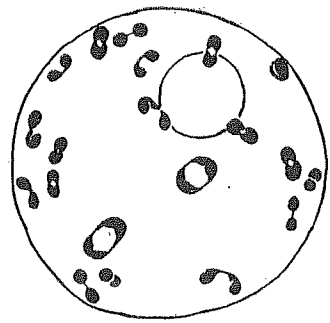
It may be noted parenthetically that the type of nucleus corresponds to that found in the Cruciferae (Manton, 1935), that is to say, it is of the "solid" type with a single central fusion-nucleolus. In organisms with large chromosomes the two or more nucleoli which are formed at telo-

phase remain distinct until the following prophase. It is possible to distinguish a polyploid from the related diploid by the number of nucleoli in the resting nuclei (*Hyacinthus* de Mol, 1928; *Crepis* Geitler, 1932). In *Aesculus carnea*, however, this is not so. At telophase several nucleoli can be seen in the process of forming, but the resting nuclei look exactly like those of its parents.

At meiosis no differences in size can be detected within the complements owing to the greater degree of contraction and the presence of chiasmata. The only difference between *Ae. Hippocastanum* and *Ae. Pavia* at diakinesis (Text-figs. 6, 7) is the difference in nuclear size. This is, however, possibly due to differences of fixation, since one was taken from a smear and the other from a section.



Text-fig. 6. *Ae. Hippocastanum*, (Smear) 33 chiasmata



Text-fig. 7. *Ae. Pavia* (Section), 27 chiasmata

Text-figs. 6, 7. Diakinesis. ×4800. Two bivalents are associated with the nucleolus in each cell.

Terminalisation is usually complete, though there is some variation from cell to cell (Text-figs. 8–11). The differences are local and affect parts of the anther and not simply individual cells. In a smear of *Ae. Pavia* I found no interstitial chiasmata (Text-fig. 10), while in one part of an anther in a section, nearly every cell had one or more (Text-fig. 11).

With slightly larger chromosomes, such as those of *Lycopersicum esculentum* or *Primula sinensis*, a distinction can be made between a loop containing two chiasmata and two free ends, but here it becomes impossible. Hence the cells with a number of apparently interstitial chiasmata may really be cells with a higher chiasma frequency, such that there are two chiasmata in one chromosome arm instead of one or none. The presence of a distal chiasma would prevent the proximal one from terminalising and would account for the difference observed. The

numbers of chiasmata have been estimated on this assumption. Since the preparations were made on separate occasions, it is probable that some external factor, such as temperature, was a cause of the difference.

Although polar views of metaphase are less valuable than side views for estimating the number of chiasmata, they nevertheless reveal relationships quite impossible to detect from side views. We see (Text-figs. 12–15 and Plate II) in each of the so-called diploids a marked degree of

Text-fig. 8. *Ae. flava* showing a ring quadrivalent, 31 chiasmata

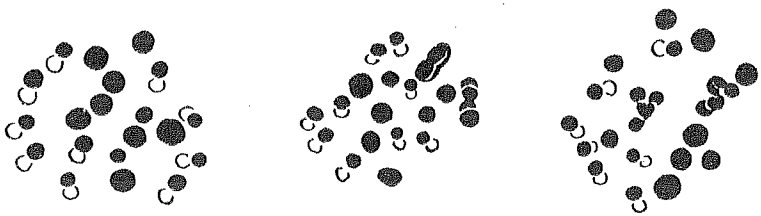
Text-fig. 9. *Ae. Hippocastanum*, 29 chiasmata

Text-fig. 10. *Ae. Pavia* (Smear), 29 chiasmata

Text-fig. 11. *Ae. Pavia* (section), 31 chiasmata. Several bivalents have two chiasmata in the same arm

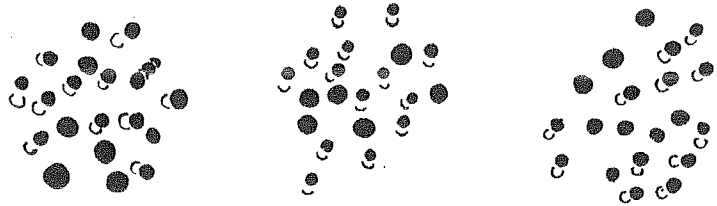
Text-figs. 8–11. Side views of first metaphase of meiosis. ×4800.

secondary pairing (Darlington, 1928). Indeed from Skovsted's illustrations, Darlington (1932 *b*, p. 218) calls *Ae. Hippocastanum* and *Ae. Pavia* "tetraploids", and *Ae. carnea* an "octoploid". The assumption of tetraploidy in the parental species is borne out by the formation of an occasional quadrivalent (Text-figs. 12, 13), not only in *Ae. Hippocastanum* but also in related species. No quadrivalents were observed in *Ae. Pavia* (Text-fig. 14), where secondary pairing is less marked (Plate II). Rather more occur in the octoploid hybrid than in *Ae. Hippocastanum*, proving that the chromosomes from *Ae. Pavia* also take part in their formation



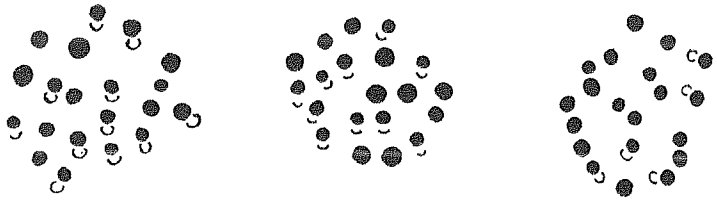
a *b* *c*

Text-fig. 12. *Ae. flava*, *a*, 10 rods, 10 rings; *b*, 2 quadrivalents, 9 rods, 7 rings; *c*, 2 quadrivalents, 7 rods, 9 rings



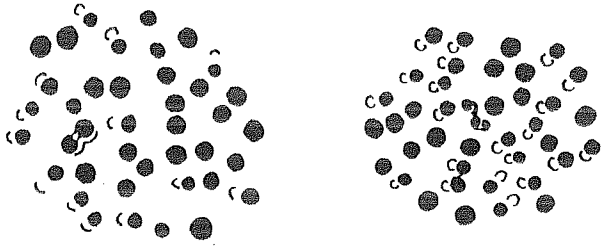
a *b* *c*

Text-fig. 13. *Ae. Hippocastanum*, *a*, 1 quadrivalent, 11 rods, 7 rings; *b*, 14 rods, 6 rings; *c*, 12 rods, 8 rings



a *b* *c*

Text-fig. 14. *Ae. Pavia*, *a*, 11 rods, 9 rings; *b*, 10 rods, 10 rings; *c*, 5 rods, 15 rings



a *b*

Text-fig. 15. *Ae. carnea*, *a*, 1 quadivalent, 13 rods, 25 rings; *b*, 2 quadrivalents, 19 rods, 17 rings

xt-figs. 12–15. Polar views of metaphase of the first division, showing secondary pairing. ×4800. The rods have one, the rings two chiasmata.

(Text-fig. 15). It is to be noted that all four chromosomes are the same size.

Polar views also cast some light on Skovsted's size differences. There are indeed size differences within the complement of *Ae. carnea* (Text-fig. 15) at this stage, as he shows, but they are due, not, as he believed, to differences in the origin of the chromosomes, since there is no difference between the parents, but to differences in the number and position of chiasmata between the bivalents (see Diagram 1). The apparently small bivalents have only one chiasma and consequently appear almost spherical. The large bivalents have two chiasmata, which bend them

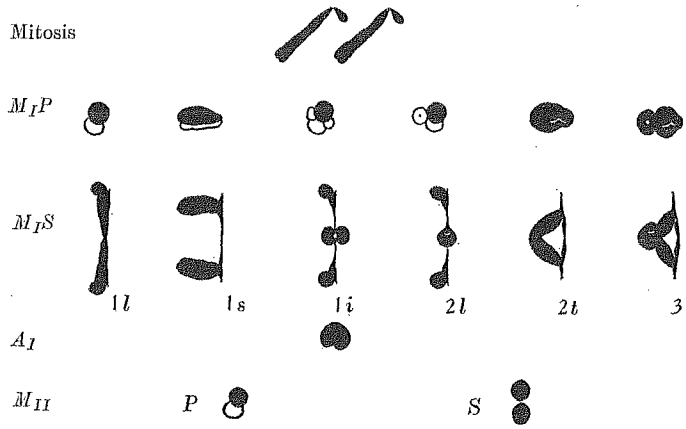
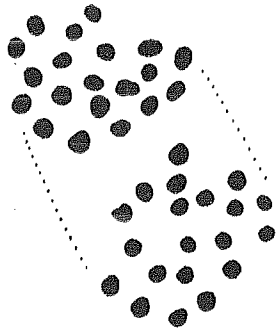


Diagram 1. Showing the effect of chiasmata on apparent chromosome size. M_I *P*, polar view of the first meiotic metaphase. M_I *S*, side view of the first meiotic metaphase. 1 *l*, one chiasma in long arm. 1 *s*, one chiasma in short arm. 1 *i*, interstitial chiasma in long arm. 2 *l*, two chiasmata in the long arm. 2 *t*, two terminal chiasmata, one in each arm. 3, three chiasmata, two in the long arm, one in the short.

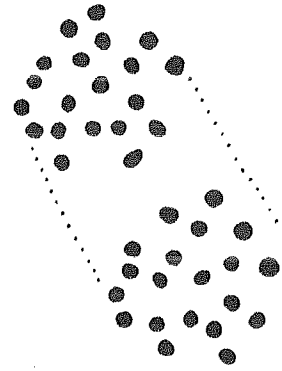
into a loop. Close examination of the plate itself shows that the "small" bivalents are on a higher and lower level, while the "large" ones occupy the middle region (cf. Text-figs. 8–11). Precisely similar differences occur in all the species.

This conclusion is borne out by a comparison of *Ae. flava* with the other forms. This species has chromosomes of the same size as its relatives, as seen at mitosis (Text-fig. 1). In polar views of metaphase of meiosis on the other hand (Text-fig. 12), it has more "large" chromosomes than the others; in side views (Text-fig. 8) it is seen to have more bivalents with two chiasmata, and consequently it forms more quadrivalents. In a word, the apparent differences in size are due to differences in chiasma frequency.

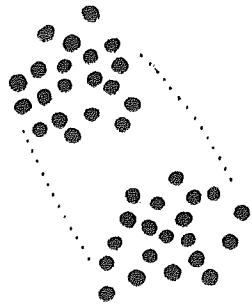
Anaphase of the first division is a more useful stage for detecting size differences, since the strain set up by the chiasmata has been released and the chromosomes are all in a similar state of relaxation as it were. The differences of size observed at mitosis are almost completely obscured



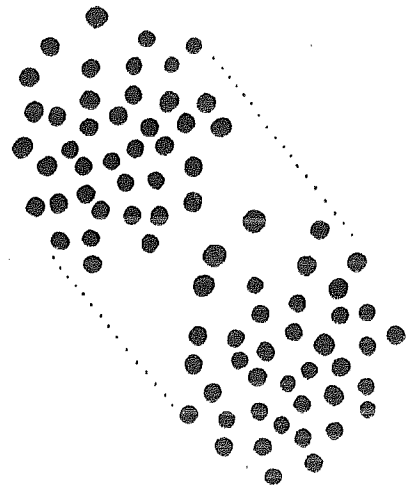
Text-fig. 16. *Ae. flava*



Text-fig. 17. *Ae. Hippocastanum*



Text-fig. 18. *Ae. Pavia*



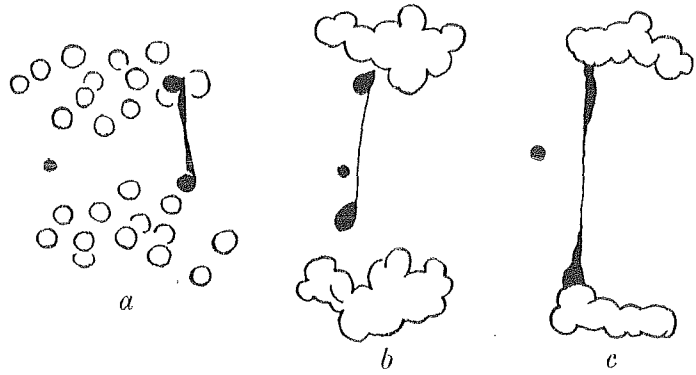
Text-fig. 19. *Ae. carnea*

Text-figs. 16–19. Polar views of anaphase of the first division. ×4800.

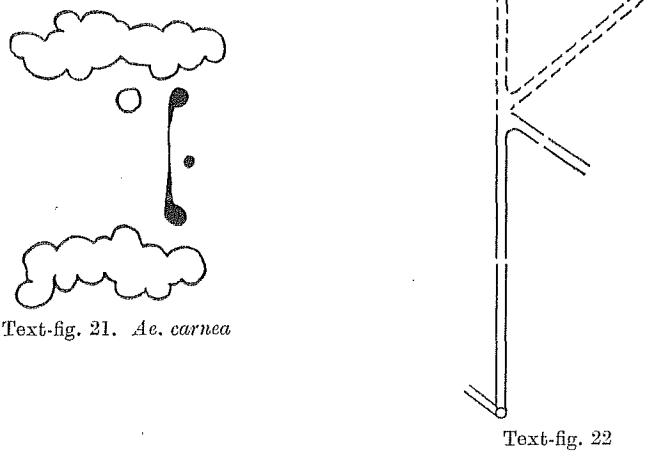
(Text-figs. 16–19). Secondary pairing is still maintained. Hoar's (1927) illustration (his Fig. 1) shows this very clearly in *Ae. carnea*.

Side views of anaphase show that the individual of *Ae. Hippocastanum* studied is structurally hybrid for part of at least one of its chromosome pairs. Somewhat infrequently, that is to say in 2 or 3 per cent. of the

anaphases observed, a bridge occurs together with a fragment, which remains lagging on the plate (Text-fig. 20). This is the result of crossing-



Text-fig. 20. *Ae. Hippocastanum*



Text-fig. 21. *Ae. carnea*

Text-fig. 22

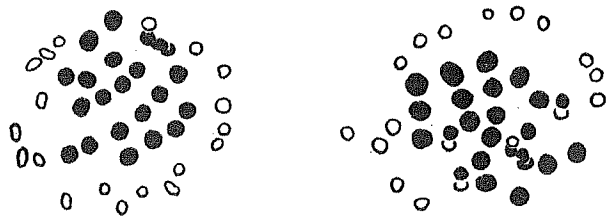
Text-figs. 20, 21. Side views of anaphase I. ×4800. Showing bridges and acentric fragments.

Text-fig. 22. Diagram showing the structure of a bivalent with a single chiasma between relatively inverted segments of homologous chromosomes (from Darlington, 1935).

over between two relatively inverted segments in such a way that of the four chromatids, one is dicentric, one acentric and two monocentric or normal (see Diagram 1). In early anaphase (Text-fig. 20 *a* and *b*) it is possible to distinguish the chromosome in which this inversion is present.

At telophase it becomes obscured among the other chromosomes, leaving only a fine thread stretching across the plate (Text-fig. 20 *c*), which is usually broken later by the formation of the cell wall. A similar bridge was found in *Ae. carnea* (Text-fig. 21), but its occurrence was rarer. It presumably results from exceptional pairing between chromosomes of *Pavia* and *Hippocastanum*.

The pairing relationships of the parental species are revealed more completely by the back-cross of *Ae. carnea* to *Ae. Hippocastanum–Ae. plantierensis*. This hybrid is triploid relative to its diploid parents, or more correctly hexaploid, with 60 chromosomes, 40 having been received immediately from *Ae. carnea* and 20 from *Ae. Hippocastanum*. Originally therefore 20 come from *Ae. Pavia* and 40 from *Ae. Hippo-*



a *b*

Text-fig. 23. Polar views of metaphase I of *Ae. plantierensis*. ×4800. *a*, 1 quadrivalent, 19 bivalents, 18 univalents; *b*, 1 quadrivalent, 21 bivalents, 14 univalents

castanum. Yet the plant does not invariably form 20 bivalents and 20 univalents. In fact I have never observed this combination. Quadrivalents occur in most cells (Text-fig. 23) and again show that the supposed diploid parents are in fact tetraploids. With them occur varying numbers of bivalents and univalents. This behaviour is an example of the well-known property of differential affinity. Chromosomes which pair regularly in the presence of an identical partner, pair variably with other and dissimilar chromosomes to which they would never be attracted in the presence of more likely mates.

A few of the univalents lie off the plate towards the poles of the spindle, but the great majority of them arrange themselves at metaphase on the edge of the plate surrounding the bivalents. This behaviour is exactly similar to that found by Kihara (1929) in the *Triticum-Aegilops* hybrids.

Chromosome measurement at mitosis and meiosis

The question of chromosome size has led investigators astray from the early days of cytology. Farmer and Digby (1914) attempted to prove that the chromosomes of the tetraploid *Primula kewensis* were at meiosis half the size of those of the diploid. Fifteen years elapsed before Newton and Pellew (1929) showed this finding to be incorrect. Again Tischler (1927) alleged that the chromosomes derived from the two parents of *Ribes Gordonianum* could be distinguished by their size at first meiotic metaphase and that there was complete autosyndesis. Darlington (1929 *a*) showed that the difference in size in the somatic divisions was not greater than that occurring within the complements of the parents, and that at meiosis normal pairing was taking place, not autosyndesis. More recently even mitosis has proved a pitfall; Ellison (1935) illustrates two varieties of potato, Golden Wonder and Langworthy, of which he says after elaborate measurement that the former has more long chromosomes than the latter. It has since been proved by Crane (1936) that Golden Wonder is a periclinal chimaera of which all but the surface layer is Langworthy. The roots of the two should therefore be identical and have been shown to be so (Upcott, *cit.* Crane, 1936).

The comparison of meiotic chromosomes in different organisms presents difficulties of an entirely different kind. The greater degree of contraction obscures slight differences of size which can be detected at mitosis. Furthermore, as we have seen, the same pair of chromosomes appears large or small according to the number and position of their chiasmata. In the genus *Aesculus* the differences within the complements of each species and of the hybrid are slight and cannot be detected at the first meiotic anaphase or at the second division. The apparent differences at the first metaphase of meiosis are due to differences in the arrangement of chiasmata and are similar in the hybrid and both the parents. It is possible that Skovsted has found a cell of *Ae. carnea* which had 20 bivalents with two chiasmata, apparently large, and 20 bivalents with one chiasma, apparently small, which he thinks have been derived from the two parents respectively.

His statement that the chromosomes of *Ae. Pavia* are larger than those of *Ae. Hippocastanum* is, however, more difficult to explain. It is possible that with his method of fixation (Carnoy) one group of species was given a different size from the other. This difference, of course, does not apply within the hybrid. That it has no validity is shown by the

uniform results I have obtained both at meiosis and mitosis in all the forms, parent, hybrid and derivative, which I have examined.

It now becomes clear that *Ae. carnea* is not an exception to the general rule of genotypic control, as it has been considered, since there is no size difference between the parents to be determined either structurally or genotypically.

SUMMARY

1. The somatic chromosomes of *Aesculus Hippocastanum* ($2n=40$) and of *Ae. Pavia* ($2n=40$) are exactly similar in size and shape, although each complement contains within it differences which are also distinguishable in the complement of the hybrid *Ae. carnea* ($2n=80$).

2. In both species, and in the hybrid, polar views of metaphase I show about half the bivalents larger than the rest, and this is due to their having chiasmata in both arms. This difference necessarily disappears at anaphase.

3. Secondary pairing and the formation of an occasional quadrivalent show the parent species to be tetraploid. The hybrid must therefore be regarded as octoploid.

4. *Ae. plantierensis* ($2n=60$) is a hexaploid back-cross of *Ae. carnea* $\times$ *Ae. Hippocastanum*, and forms varying numbers of multivalents, bivalents and univalents.

5. The individuals of *Ae. Hippocastanum* and *Ae. carnea* examined are heterozygous for inversions.

ACKNOWLEDGMENT

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EXPLANATION OF PLATES I AND II

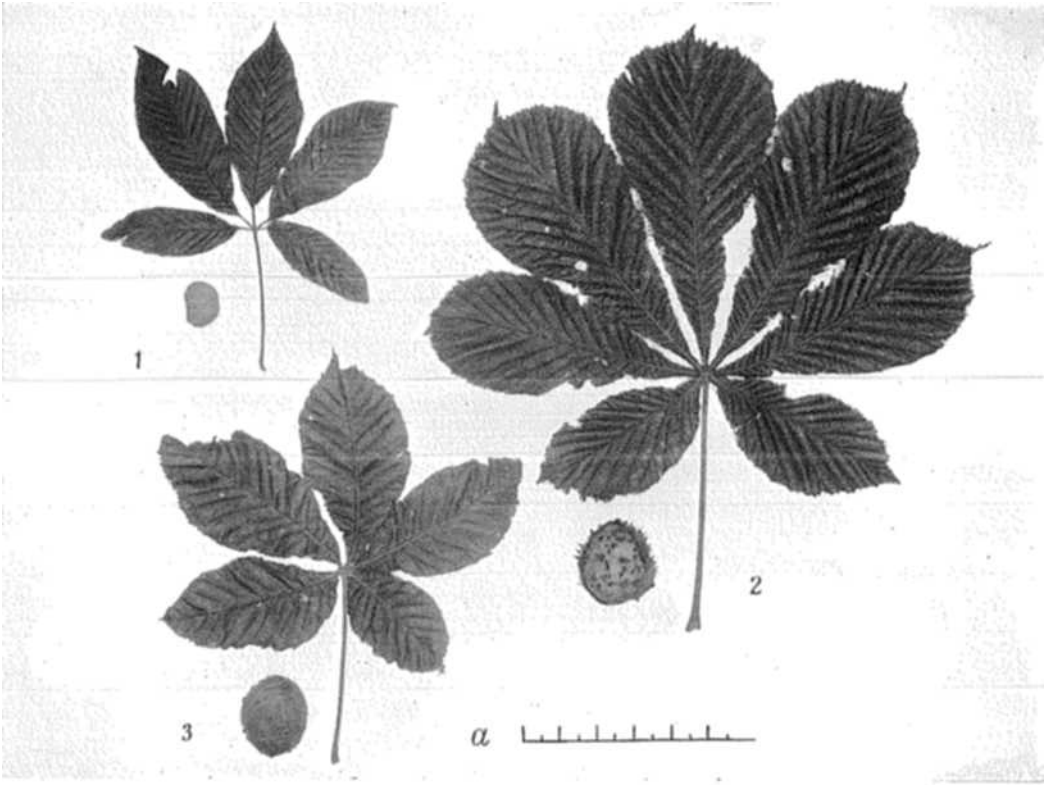
PLATE I

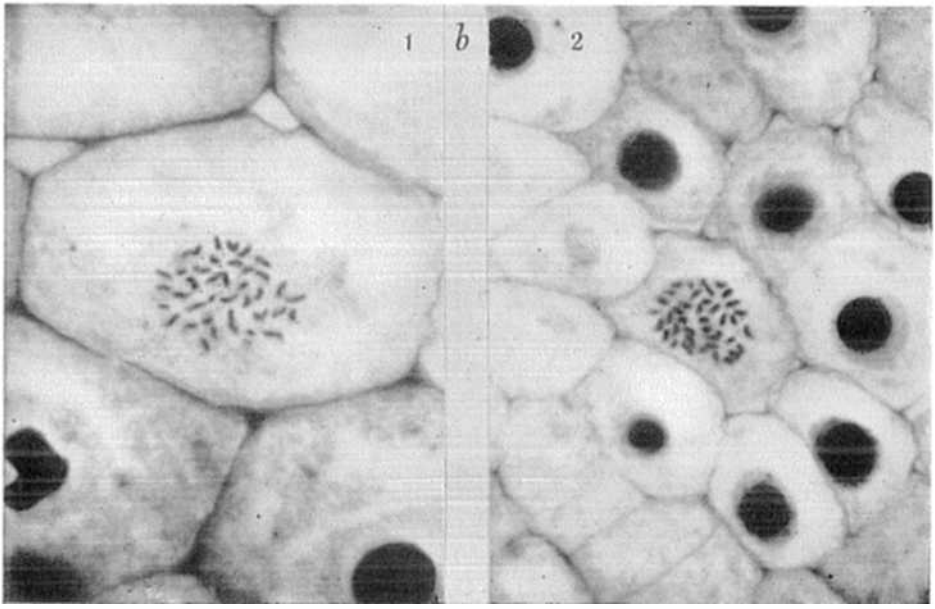
(*a*) Leaves and fruit of the parents and hybrid showing the intermediate character of the latter. (1) *Ae. Pavia*; (2) *Ae. Hippocastanum*; (3) *Ae. carnea*.

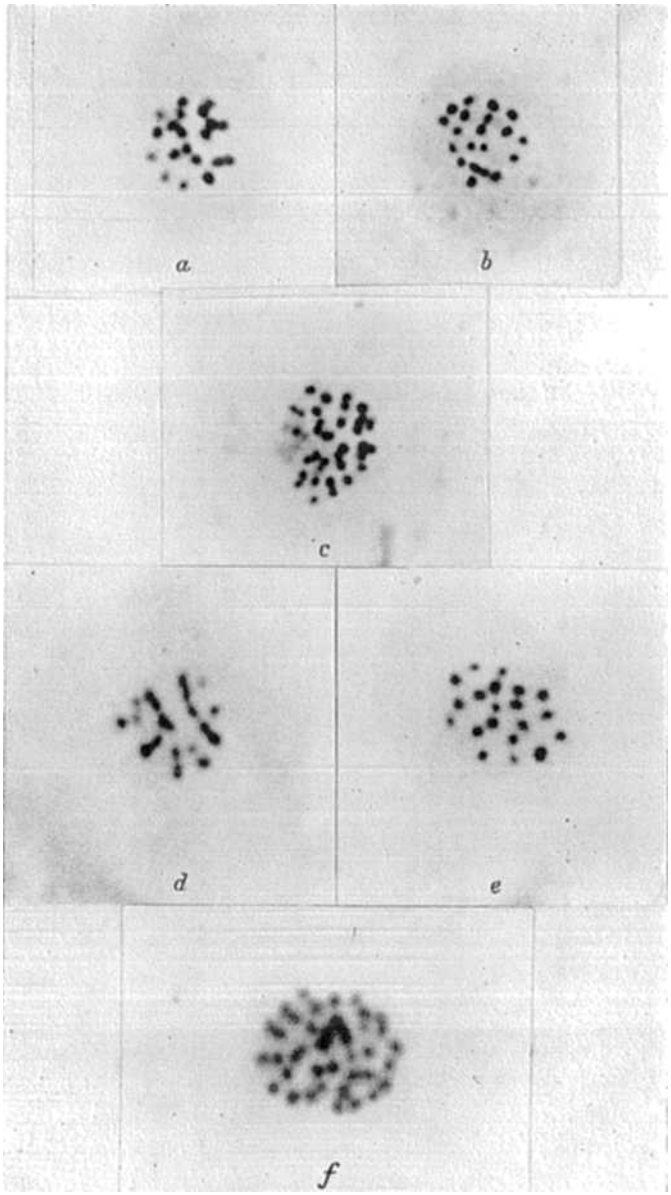
(*b*) Somatic divisions from the root tip of (1) *Ae. Hippocastanum*, (2) *Ae. Pavia*. ×2400. The "solid" nuclei and prochromosomes can also be seen.

PLATE II

Polar views of first metaphase showing secondary pairing. (*a*) *Ae. Hippocastanum*; (*b*) *Ae. Pavia*; (*c*) *Ae. carnea*; ×2400. (*d*) *Ae. Hippocastanum*; (*e*) *Ae. Pavia*; (*f*) *Ae. carnea*; ×3200.


1
2
3
a


1
b
2


a
b
c
d
e
f

APPENDIX

Since sending this article to the press, I have been able to compare the *Ae. Pavia* plants from the Royal Gardens, Windsor, and from the University Botanic Garden, Copenhagen, with the descriptions and illustrations in:

Loddiges Botanical Cabinet, **13**, 1257.

Engler and Prantl, *Pflanzenfamilien*, III, **5**, 276.

Britton and Brown, *Illustrated Flora of the Northern United States*, etc., **2**, 500.

Rehder, *Manual of cultivated trees and shrubs*, p. 582.

The Windsor plant which I used is true *Ae. Pavia*. The Copenhagen plant, used by Skovsted, is *Ae. hybrida*, a hybrid between *Ae. octandra* and *Ae. Pavia*.

I am indebted to Mr Cook of the Royal Gardens at Windsor and to Dr Axel Lange, Curator of the Copenhagen Botanic Garden, for sending me seeds and flowers.