tracts from neurosecretory cells of the brain were submitted to a similar heat treatment, little or no activity was lost. Since this difference might have been due to the much smaller amount of protein precipitating from brain-cell extracts than from blood, the experiment was repeated with blood samples which had been diluted ten times, and MNC extracts prepared in Ringer solution to which 10 percent inactive blood had been added. The same difference in temperature lability was observed. This would indicate that bursicon in the blood is much less heat stable than the hormone in the MNC. Extracts from the ganglion behaved the same way as neurosecretory cell extracts.

The hormone activity of the blood was nondialyzable and was precipitated by $(NH_4)_2SO_4$ without loss of activity (*4*). Extracts from the neurosecretory cells and the ganglion reacted essentially in the same way. These experiments, together with the results from gel-filtration and electrophoresis, dispose of the idea that the hormone in the brain might be chemically and functionally entirely different from bursicon and act merely as a trigger for the release of bursicon from the ganglion, in the same sense as a brain hormone releases ecdyson from the prothoracic glands. The hormone in brain and ganglion shows almost the same properties as that in the blood, the only noticeable difference being the greater lability to heating in the blood and the appearance of a second peak of activity on electrophoresis of extracts from brain cells. In our present stage of knowledge it would be futile to attempt an interpretation of these differences.

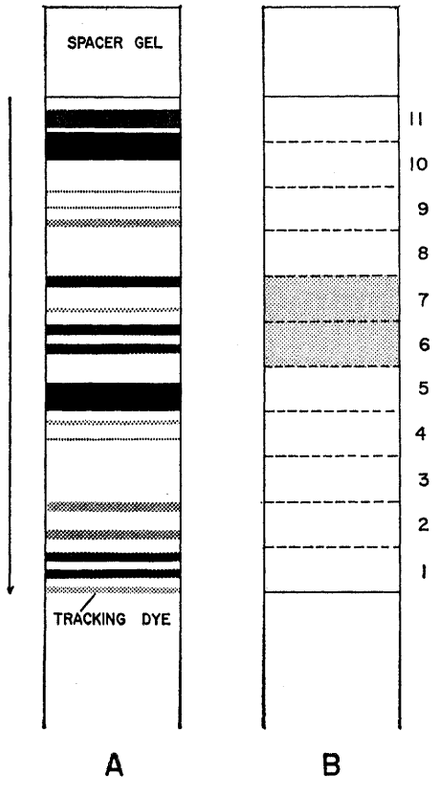

Fig. 3. (*A*) Interpretation of disc electrophoresis pattern of active blood (Fig. 2*A*). (*B*) Location of bursicon activity on the column after electrophoresis of active blood. The material moves in the direction of the arrow toward the anode.

Bursicon activity was also demonstrated in the blood of the roach *Periplaneta americana* at the time of emergence, and in the ganglia and corpora cardiaca at all times (*4*). Recently it was reported that the last abdominal ganglion of roaches plays a special role in tanning (*10*). These, and similar observations with other insects (*4*), have suggested that the tanning hormone of insects is unspecific. A comparison was made of active fractions from the blood, last abdominal ganglion, and corpora cardiaca from *P. americana* with corresponding fractions from flies. The relatively low hormone activity in roach blood has so far precluded the use of gel-filtration with its concomitant dilution of activity. Electrophoresis of roach blood, for reasons so far unexplained, did not lead to a clear-cut separation of hormonal activity. The active constituent from the last abdominal roach ganglion migrated in electrophoresis to the same locations 6–7 as with the various fly preparations (Table 1). Extracts from the corpora cardiaca showed exactly the same division of peaks of activity into two fractions (Table 1) as reported above for the neurosecretory cells of the fly. These results quite unexpectedly illuminate and support the fact that the corpora cardiaca constitute a storage organ for the brain hormone (*11*).

Evidence of protein nature has been claimed before, on the basis of reactions to precipitating agents, dialysis, and proteases, both for a brain hormone of the pupa of *Phi'osamia* (*12*) and for a diabetogenic hormone in the crustacean eyestalk (*13*).

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References and Notes

1. C. B. Cottrell, *J. Exp. Biol.* **39**, 395, 413, 431, 449 (1962).
2. G. Fraenkel and C. Hsiao, *Science* **138**, 27 (1962).
3. ———, *ibid.* **141**, 1057 (1963).
4. ———, *J. Insect Physiol.* **11**, 513 (1965).
5. Bio-gel P. Instruction sheets issued by Calbiochem.
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
7. A. D. Anderson and R. B. March, *Can. J. Zool.* **34**, 68 (1965).
8. P. Andrews, *Biochem. J.* **91**, 222 (1964).
9. L. Ornstein and B. J. Davies, *Disc Electrophoresis* (Distillation Product Industries, Rochester, N.Y., 1962).
10. R. R. Mills, R. B. Mathur, A. A. Guerra, *J. Insect Physiol.* **11**, 1047 (1965); R. R. Mills, *ibid.*, p. 1269.
11. E. Scharrer and B. Scharrer, *Neuroendocrinology* (Columbia Univ. Press, New York, 1964).
12. M. Ichikawa and H. Ishizaki, *Nature* **198**, 308 (1963).
13. L. H. Kleinholz, H. Esper, C. Johnson and F. Kimball, *Amer. Zool.* **1**, 366 (1961), and personal communication by Kleinholz.
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Chromosome Complement: A Fertile Hybrid between Equus prjewalskii and Equus caballus

Abstract. *At the Zoological Garden of Antwerp it has been proved that when* Equus prjewalskii *is crossed with* E. caballus *the offspring is fertile. As expected, its diploid chromosome number is 65. The morphological differences between the parental and the offspring karyotypes are not great; therefore, meiosis in the hybrid was successfully achieved.*

A difference between the diploid chromosome numbers of *Equus caballus* ($2N = 64$) and *E. prjewalskii* ($2N = 66$) has recently been reported (*1*). For a long time, one of us was of the opinion that *E. caballus* and *E. prjewalskii* in fact represented two different species of the horse family. As no living hybrids were known to exist, experimental breeding between Prjewalski's horse and the domestic mare was undertaken at the Zoological Garden of Antwerp. Two races of *E. caballus*, the Norwegian fjord pony and the Pyrenees pony, had been selected for this purpose since they were geographically the most distant from Mongolia, the habitat of *E. prjewalskii*. Further experiments proved that one of the two resulting male hybrids was fertile (*2*). This stallion, born in March 1962, impregnated late in 1964 a female Pyrenees pony, which gave

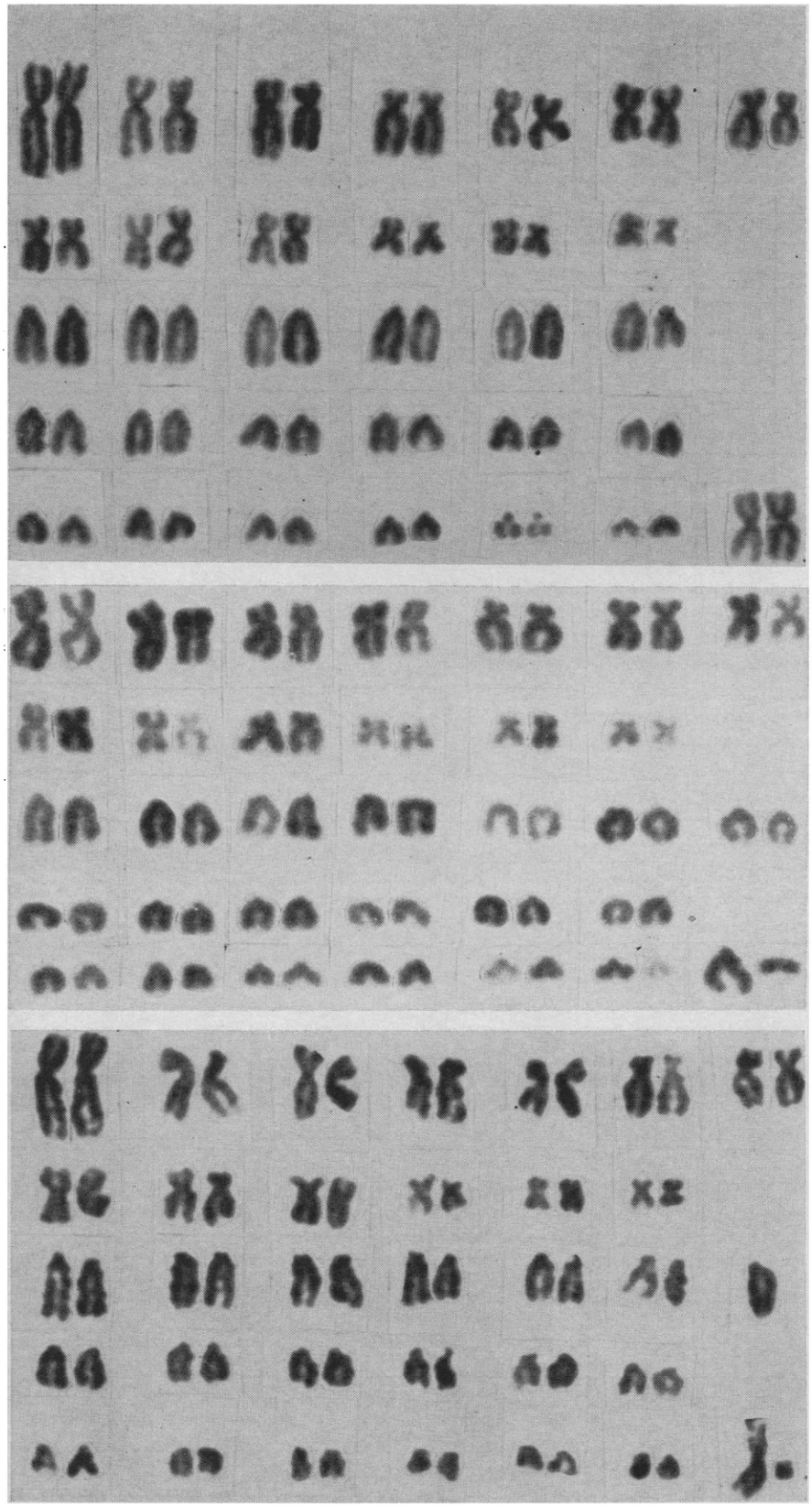

Fig. 1 (Top). Karyotype of a metaphase from the Norwegian fjord pony. (Middle) Karyotype of a metaphase from *E. prjewalskii*. (Bottom) Karyotype of a metaphase from the hybrid. The metacentric chromosomes are ranged in the first two rows. Their morphology and number are identical for the three animals. The acrocentric chromosomes are in the last two rows. Prjewalski's horse has two more of these, and the hybrid has one more, than the domestic horse. The sexual chromosomes are at the right of the last acrocentric row.

birth to a female "¼ of hybrid." We then undertook karyotype studies of the fertile hybrid to obtain indirect confirmation of Benirschke's results and to assess probable reasons for the animal's fertility. However, for completeness, not only the chromosome complement of the hybrid, but also the karyotypes of its parents, a male Prjewalski's horse [Ludwig-Koedie studbook number 87 (*3*)] and a female Norwegian fjord pony, were examined.

We used blood cultures according to the method of Moorhead *et al.* (*4*). Results are reported in Table 1. The diploid number of 66 for *E. prjewalskii* was confirmed, directly and indirectly, by the fact that the hybrid has 65 chromosomes. The Norwegian fjord pony has a diploid number of 64, as do all other specimens of *E. caballus*.

In the absence of a model of karyotype for the horse (*1*), we followed Benirschke's classification and grouped the chromosomes into two series, the metacentrics and the acrocentrics. Fifteen karyotypes per horse were reconstructed. A model for each specimen is proposed (Fig. 1). The number and the morphology of the metacentric autosomes seem identical for the three species. The variations in the diploid numbers are due to the acrocentric chromosomes—there are two more in *E. prjewalskii* and one more in the hybrid than in *E. caballus*. The general trend of a correlation between a high diploid number and a high number of acrocentrics has been found (*6*). However, a Robertsonian reduction of the total number of chromosomes from the "primitive" to the recent form is not apparent. Indeed, only a study of meiotic figures would elucidate this question definitely, and only such a study would permit the formation either of an autosomal trivalent or of an autosomal univalent to be observed. Unfortunately, fragments of the hybrid's gonads are presently not available. Nevertheless, we believe that our findings could account for the fertility of the hybrid; achievement of meiosis seems to be possible without important chromosomal rearrangements.

Our breeding program is in progress, along with somatic cytogenetic studies. The chromosomes of the offspring of the hybrid ($2N = 65$) and *E. prjewalskii* ($2N = 66$) will be of interest to investigate. We will be able to observe a horse with 66

Table 1. Distribution of chromosome numbers in analyzed metaphases for each horse studied.

62	63	64	65	66	67
Norwegian fjord pony ♀					
1	1	48			
Prjewalski's horse ♂					
		1	1	37	1
Hybrid ♂					
	2	5	43		

chromosomes and a karyotype morphologically very similar to (if not identical with) the karyotype of *E. prjewalskii,* despite the fact that this animal may not be a pure Prjewalski's horse. This situation indeed would raise questions about the specificity of karyotype studies of specimens from zoological families with living, fertile hybrids.

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References and Notes

1. K. Benirschke, N. Malouf, R. J. Low, H. Heck, *Science* **148**, 382 (1965).
2. S. Frechkop, *Bull. Inst. Roy. Sci. Nat. Belg.* **40**, 1 (1964).
3. J. Volf, *Pedigree Book of the Prjewalskii Horse* (Zoological Garden, Prague, 1961).
4. P. S. Moorhead, P. C. Nowell, W. S. Mellman, D. M. Battips, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960). The cultures were set up in the Centre de Cytogénétique de la Faculté de Médecine de l'Université Libre de Bruxelles. We thank Prof. Twiesselmann for his hospitality.
5. J. L. Hamerton, H. P. Klinger, D. E. Mutton, E. M. Lang, *Cytogenetics* **2**, 240 (1963).
6. We thank Dr. W. Vandenbergh, director, and Prof. Mortelmans, Veterinarian, at the Zoological Garden of Antwerp, for their help.
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Photoperiodic Induction of Senescence in Xanthium Plants

Abstract. Xanthium *plants placed under photoinductive conditions were accelerated in their senescence whether or not the plants had a capacity to produce flowers and fruits. Thus, the role of the mobilization influence of these organs on the senescence of certain monocarpic plants can be questioned.*

The rapid decline of vegetative growth and the dramatic onset of senescence in many annual plants have been attributed to the mobilizing influence of developing flowers and fruits (*1*). The inadequacy of this hypothesis to fully explain senescence in all monocarpic plants (those that form seeds once and die) has been shown in experiments with soybean (*2*), Alaska pea (*3*), and cotton (*4*), in which removal of flowers and fruits failed to prevent cessation of growth or to extend the life of the plants for more than a limited time. Further evidence against a simple mobilization basis of senescence is seen from studies on dioecious plants, for example, hemp (*5*) and spinach (*6*), in which the development of small staminate inflorescences is sufficient to induce senescence.

Although there is considerable evidence for the regulatory function of day length in controlling bud dormancy (*7, 8*) and leaf senescence (*7, 9*) in woody plants, the exact role of photoperiodic induction in the aging process of herbaceous plants has not been established, mainly because few investigators have studied senescence in photoperiodically sensitive species. Where these plants have been used, the influence of flower and fruit development generally has not been considered separately from that of photoinductive treatment alone.

Effects of flower and fruit development on senescence may be eliminated by using completely debudded plants, that is, those having both terminal and lateral buds removed. That the metabolic changes accompanying photoinduction may be similar in debudded and intact plants was shown by Vergara and McIlrath (*10*) in their studies on the influence of photoperiod on water-absorption by the short-day plant *Xanthium pensylvanicum.* Similar shifts in uptake of water were observed in both debudded and intact photoinduced plants even though the debudded plants never attained the developmental stages of synapsis, syngamy, and fruit development. These findings suggested that the effects of short-day treatment on the induction of senescence in debudded *Xanthium* plants should be explored.

Although our experiments are preliminary, the results obtained were reproducible and, therefore, provide further evidence that the death of certain annual plants is independent of the developmental events of flower and fruit production.

Experiments were carried out in the greenhouses at the University of Chicago. Burs of the Chicago strain of cocklebur (*Xanthium pensylvanicum* Wallr.) were soaked in tap water for 2 days in a wide-mouth bottle; the water was then displaced with oxygen and the bottle stoppered. After 24 hours the burs were planted in flats of vermiculite. When the seedlings had formed two true leaves, they were transplanted to 13-cm pots of fertile soil and placed on the greenhouse benches under a 20-hour (20H) noninductive photoperiod (consisting of 20 hours of light and 4 hours of darkness per 24-hour period). The plants were maintained in this environment for approximately 6 weeks.

Table 1. Effect of time of bud removal on senescence of *Xanthium* plants. 20H, noninductive photoperiod consisting of 20 hours of light and 4 hours of darkness in a 24-hour period; 9H, inductive photoperiod consisting of 9 hours of light followed by 15 hours of darkness.

Photoperiod	Mean days to death*	Range in days to death
Nondebudded		
20H	No death†	
9H	66.0	63 to 70
Debudded when photoperiodic treatment began		
20H	112.0	91 to 118
9H	64.7	63 to 66
Debudded 16 days later		
20H	130.6	118 to 139
9H	65.1	63 to 66

* Average number of days from start of photoperiodic treatment to death of the plant. Ten plants per treatment. † Nondebudded plants still alive after 365 days on 20-hour treatment.

Plants on the photoperiod trucks and greenhouse benches received 8 hours of natural light. Additional light to extend the photoperiod or interrupt the dark period was provided by 200-watt incandescent bulbs (providing approximately 1650 lu/m^2 at the level of the plants).

Plants were debudded by removing both terminal and lateral buds; the terminal buds were excised when the sixth leaf produced by the plants was at least 1 cm in length and the lateral ones were excised as soon as they were large enough to be successfully removed. The date on which all leaves on the main stem were completely dehydrated was recorded as the time of death of the plant. Ten plants were used per treatment.

In the first experiment, comparable sets of debudded and intact plants were either transferred to a continuous 9-hour (9H) inductive photoperiod (9 hours of light followed by 15 hours of darkness) or kept under continuous long-day (20H) conditions. Debudded photoinduced plants exhibited many of the same senile changes ob-