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With 1 *Plate*
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Cytological observations on a duck hybrid: *Anas clypeata* × *Anas penelope*

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1. INTRODUCTION

Generic hybrids in ducks have been studied cytologically by Sokolowskaja (1935), Crew & Koller (1936) and Yamashina (1941). In all these cases *Anas platyrrhynchos* and *Cairina moschata* were used as parental forms and the gonads, mostly testes of the invariably sterile hybrids, were ctyologically analysed and compared with parental forms.

The present paper deals with a species cross in which the parental forms were: *Anas* (*Mareca*) *penelope*, wigeon, as the male parent and *Anas* (*Spatula*) *clypeata*, shoveler, as the female parent. Apparently there was some doubt about the taxonomic position: whether wigeon and shoveler form two distinct genera or only two different species of the same genus; the latter opinion seems to prevail.

2. MATERIAL AND METHODS

This work was done in collaboration with Dr J. M. Harrison of Sevenoaks in Kent. The morphology of a sibling of the present hybrid was described in detail and compared with the parental forms by Dr Harrison (1959). For the present study the male birds of the parental forms and one male hybrid were injected by Dr Harrison with colcemid 2 hours prior to killing. All birds were adult, the hybrid was 5 years old; their weight was between 680–780 gr. The birds were killed on the following dates: wigeon on 11th of May, hybrid on 18th and shoveler on 19th of May 1963. The testes were removed and divided into two parts. One part was treated with a hypotonic solution of sodium citrate before fixing in acetocarmine, the other was fixed directly. Within 3–4 days the samples arrived in Edinburgh and preparations were made using acetic orcein, lactic orcein or basic fuchsin. The slides were made by squash and smear techniques and also from paraffin sections.

3. RESULTS

In mammals the seminiferous tubules show some variation in diameter. This variation is very probably related to the phase of the spermatogenetic wave. For instance in the mouse first meiotic divisions are usually abundant in those parts of the tubules in which their diameter undergoes rapid change.

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In both parental forms studied in this paper the diameter in the narrow region of the tubule is to that in the wide regions as 1:1½. In the hybrid this ratio becomes 1:3 or even greater (Plate I, Fig. 1 s, w, h). On the whole the wigeon has much thinner tubules and they show considerable variation in their diameter. The shoveler has tubules of a greater and more uniform diameter. The hybrid tubules exceed those of the shoveler in thickness but do not fall below those of the wigeon in minimum diameter.

The changes of the diameter in the hybrid and in both parental forms are not regionally fixed; they are local and temporary expansions, due probably to the rapid increase in the number of cellular elements contained in the tubule. This opinion is supported by the fact that in narrow parts of the tubule the nuclei of the cells of the *membrana propria* are separated by smaller distances than in the expanded parts. In the hybrid the nuclei of these cells are not similarly crowded in the narrow parts, indicating that the change of diameter in the hybrid is not entirely due to the phase of the spermatogenetic wave. It appears that in the hybrid the narrow regions represent segments of the tubules with reduced (or completely absent) meitotic activity (Plate I, Fig. 2 s, w, h). It is interesting to note that the size of cells and of nuclei in the hybrid is greater than in both parental forms; this was observed both for spermatogonial cells and for auxocytes. When the average diameter of the nucleus is taken as a measure, then the increasing order of nuclear sizes is: wigeon, shoveler, hybrid.

However, with only one individual of each type it is difficult to know whether all these differences are characteristic of the three types or whether some of them result from chance differences. There are enormous seasonal differences in testis size in ducks, but it is not known how much and how quickly they affect the diameter of the tubules or the size of cells and of nuclei. It is doubtful whether this

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EXPLANATION OF PLATE

Fig. 1. Tubuli seminiferi of the teased testes, s—shoveler, w—wigeon, h—hybrid. Aceto-carmine stained unsquashed material.

Fig. 2. Cross sections of the tubuli seminiferi, s, w, h, as above. Paraffin sections 10 μ thick. Feulgen stain. In s, spermateleosis is well advanced in the thicker part of the tubule and less so in the thinner part on the right. The two sections represent different phases of spermatogenetic wave. In w the thicker part is at the beginning of sperm differentiation. In h the thicker tubule contains spermatocytes of the second order, the thinner tubule on the right appears to have reduced or absent meiotic activity.

Fig. 3. Spermatogonial metaphase plates. s, w, h, as above, d—metaphase plate of a hybrid showing slight morphological differences in the pair of longest chromosomes at the right-hand side of the photograph.

Fig. 4. Pachytene chromosomes, s, w, h, as above. In w there are much fewer elements than in s. In h non-paired middle regions of the chromosome. d—chain of short chromosomes in the late pachytene stage. Basic Fuchsin staining.

Fig. 5. Diplotene/diakinesis, s and w show chiasmata in many chromosomes, in h non-pairing regions visible in many chromosomes.

Fig. 6. First meiotic metaphase in the hybrid: (*a*) polar view showing few univalents, (*b*) side view showing assymetrical segregation.

Scale of magnification for 1 10× for 2 100×, for the remaining photographs about 900×.

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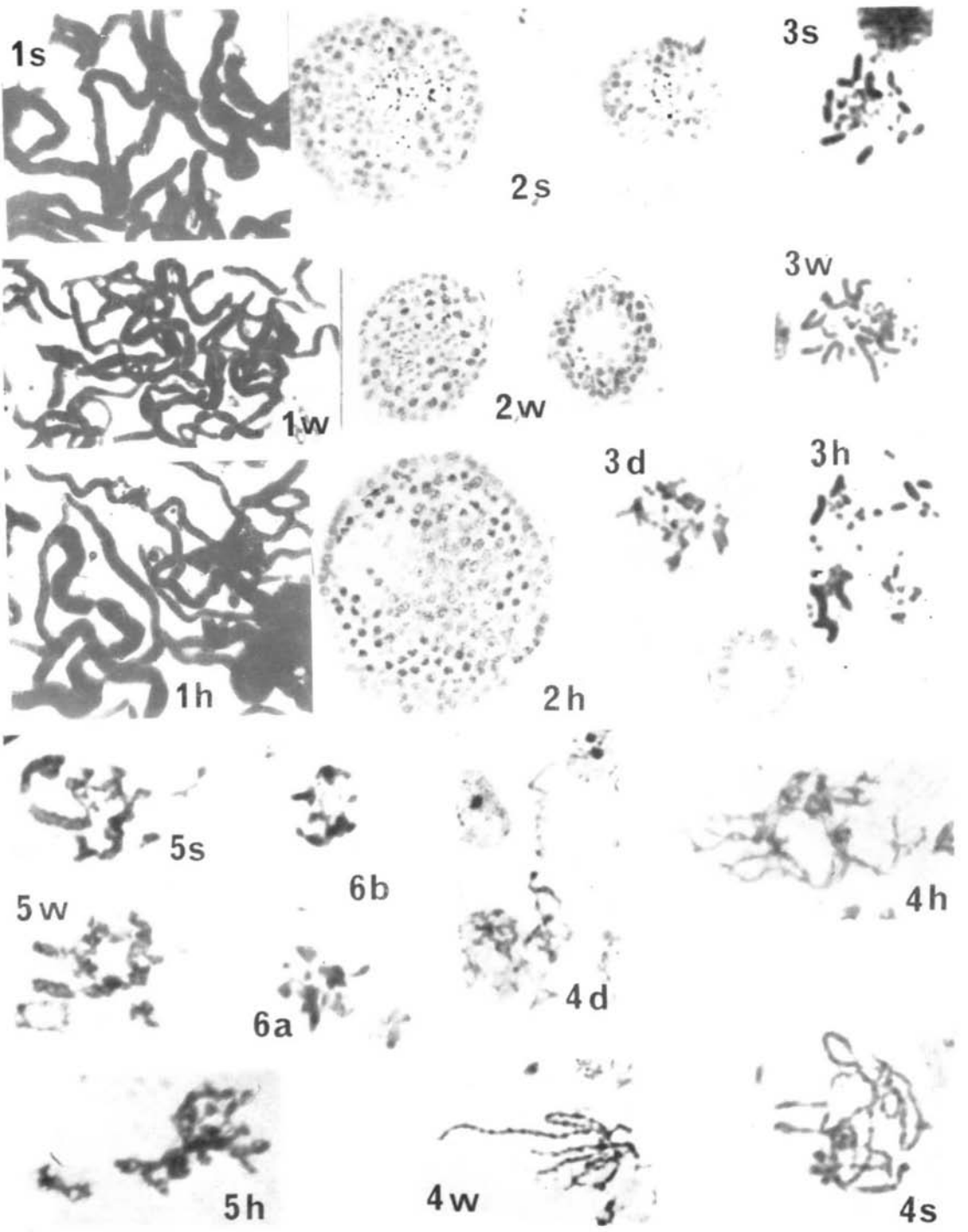
Plate I Figs. 1–6



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seasonal variation could be detectable in the present material in view of the fact that all three birds were killed within a week.

As is usual in avian cytology, the number of separate chromosomes visible as distinct units in spermatogonial metaphase plates could not be established. It appears that in addition to six pairs of large chromosomes, found by all authors who studied the genus *Anas*, there is a great number of short chromosomes which could not be satisfactorily counted. Sokolowskaja (1935) counted about 38, Crew & Koller 60 and Yamashina 68 of these short elements. The number of short elements in the somatic metaphase plate in both parental forms and in the hybrid is very probably nearer the latter figure.

The large chromosomes of both parental forms and of the hybrid seem to be very similar in their morphological features, although a possibility of some slight differences cannot be excluded. Some indication for this suggestion is shown in the spermatogonial metaphase plate of the hybrid (Plate I, Fig. 3 d), where on the extreme right side of the photograph there are two undoubtedly homologous chromosomes lying side by side, one of which is not only smaller but also has a relatively shorter long arm. A similar phenomenon was described by Yamashina (1941) in his study of the intergeneric hybrid between *Anas* and *Cairina*.

In the hybrid described here, pachytene pairing of long and of short chromosomes seem to be irregular. There are regions of the chromosomes which are very far apart as if they were actually repelling each other, although they are included between two strongly paired end segments. This gives a peculiar looped configuration to the pachytene bivalents of the hybrid (Plate I, Fig. 4 h), quite different from the usual appearance of chiasmata alternating with non-paired regions.

The number of distinct chromosomal elements counted in the pachytene nuclei varies from 24–38. The length of the longest pachytene element shows also variation, the ratio between its minimum and maximum length being in extreme cases $1:1\frac{3}{4}$ (Plate I, Fig. 4 s, w. h).

In the hybrid the pachytene chromosomes sometimes assume chain-like configurations which consist of end-to-end association of bivalents. When more chromosomes combine in this way, a zigzag configuration is obtained as shown in Plate I, Fig. 4 d.

The next stages of meiotic prophase, diplotene and diakinesis, appear to proceed normally in both parental forms, while in the hybrid the bivalents show loops of repelling regions similar to those observed in pachytene: the homologous chromosomes are held together at both ends, their middle segments being very far apart. It is not known for certain what happens to these figures in metaphase (Plate I, Fig. 5 s, w, h). It is possible that the loops are closed as the chromosomes condense and their charge of stainable substances increases.

In some cases (less than 1%) univalents were seen in the hybrid suggesting that there may be some differences in homology, or in the timing of the pairing. Sometimes the first anaphase separation in the hybrid shows some disturbances which probably lead to numerical abnormalities in the daughter nuclei. The frequency of these abnormalities is however exceedingly small (Plate I, Fig. 6 a, b).

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4. DISCUSSION

In their study of intergeneric hybrids in ducks Crew & Koller (1936) observed that the chromosomes as a rule paired more or less normally, but that the spindle mechanism did not work properly. The authors ascribed the sterility of the hybrids between *Anas platyrrhynchos* and *Cairina moschata* largely to the abnormalities of the spindle. Yamashina (1941) in an identical hybrid found the meiotic phenomena to be highly irregular, but he considered the failure of pairing to be the main cause of hybrid sterility, while the spindle mechanism in his material was apparently quite normal. Both these possible mechanical causes of intergeneric sterility are cytologically recognizable in rudimentary form in the species hybrid described here. There is however no information about its fertility.

The failure of pairing recorded here is probably different from that described in the generic hybrid by Yamashina (1941). The failure of pairing in the species hybrid does not affect the whole length of the chromosomes in question. As mentioned above both ends of the homologous chromosomes are associated while their midparts appear to repel each other. This is then a partial failure of pairing unlike the complete one of the Japanese author.

In the absence of pairing in the midparts of the chromosomes, the excess of stainable substance accumulated on the ends of the chromosomes causes the corresponding chromosomes to find each other and to form a pair; this in turn results in symmetrical segregation and numerical constancy. Thus the stainable substance appears to be the pair-forming agent even in the absence of strict homology and consequently proper segregation can follow in the absence of chiasmata.

In this respect the conditions in the hybrid chromosomes are similar to those in *Drosophila melanogaster* males, where excess of the stainable substance of the chromosomes, particularly at their ends, ensures pairing and symmetrical segregation even in partly non-homologous translocation bivalents where chiasmata are not formed (Slizynski, 1964). The fundamental causes for the absence of chiasmata in the normal male flies are not known. In the hybrid described here the possible absence of chiasmata may be due to the total or partial loss of homology. In both cases it is the stainable substance of the chromosomes which prevents the disintegration of the karyotype in meiosis.

In the opinion of the present author the failure of pairing in the hybrid indicates partial non-homology. The other possibility, that the non-paired regions result from inversions, can be ruled out in view of the great similarity of the pachytene pattern and of the absence of inverted pairing. In the latter case the midpart of the chromosome is paired, the free ends remaining loose. That is, assuming that pairing may start anywhere, besides association of the type: $\frac{a}{a}\frac{b}{b}\frac{c}{e}\frac{d}{d}\frac{e}{c}\frac{f}{f}\frac{g}{g}$, (where the two ends pair), the opposite configuration: $\frac{a}{g}\frac{b}{f}\frac{c}{c}\frac{d}{d}\frac{e}{e}\frac{f}{b}\frac{g}{a}$ should also be present. This however is not the case; inverted pairing and inversion loops have never been found.

There can be no doubt that the accumulation of gene mutations brings about

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the non-homology which may lead to the species divergence. Similar conclusions were reached by Matthey & Van Brink (1960) who, on the basis of morphological similarity of chromosomes of related species, stated that the accumulation of gene mutations is more important for evolution than structural alterations of the chromosomes. This appears to be so for the Vertebrates, where inversions are exceedingly rare. It is not so for the genus *Drosophila*, where inversions have played an important role in the evolution of some species. The difference may be due to some fundamental properties of the vertebrate chromosomes as, e.g. functional polarity, the formation of which is indicated by the findings of Gall & Callan (1962). Functional polarity would prevent inversions.

Stenius, Christian & Ohno (1963) compared the karyotype of *Phasianus colchicus*, *Gallus domesticus* and *Meleagris gallopavo*. They found that while *Gallus* and *Phasianus* from the same family show important differences in chromosome morphology, there is no detectable difference between the chromosomes of *Phasianus* and *Meleagris gallopavo*, which are species belonging to two distinct families. The authors conclude that during the evolution of these two species from an ancestral form of the order *Galli*, the original set of chromosomes remained relatively unchanged.

From a consideration of the hybrid described here it is apparent that the first stages of species divergence are based on gene mutations only, the karyotype not undergoing change, if the smaller size of one chromosome (Plate I, Fig. 3 d) is disregarded.

Class *Aves* is characterized cytologically by great uniformity of the karyotype in all its taxonomical groups. All birds so far studied contain few (6–8) pairs of relatively large chromosomes (including the sex chromosomes) called by Matthey macro-chromosomes (1949), and a varying number (up to 40 pairs) of short dot-like chromosomes, microchromosomes of Matthey. In this respect the birds are similar both to the Reptiles and to the Monotremes.

The chromosomal nature of the short chromosomes has been doubted. Newcomer (1963) considers them to be acentric, variably heterochromatic, prone to fusion and fragmentation, and disappearing in the second meiotic division of spermatogenesis. In addition, he describes an asynchrony in DNA synthesis between the large chromosomes and the 'chromosomoids', as he calls the microchromosomes. In defence of the microchromosomes being true chromosomes Ohno (1960) and Ohno *et al.* (1962) bring the following evidence: in insufficiently fixed metaphase nuclei, microchromosomes may become sticky and appear to aggregate. There is also the fact that they can be individually recognized from the early stages of prophase, and that they do take part in nucleolus organization.

The present study revealed that in a well-spread pachytene chromosome complement the number of separate elements varies and that the nuclei with a smaller number of these contain longer chromosomes (Plate I, Fig. 4 s, w, h). This indicates that in meiotic prophase, chromosomes may enter into a temporary end-to-end association. This assumption was supported by the observation that the length-wise association of pachytene chromosomes could be recognized by their chromomeric pattern.

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If the breaking up of the end-to-end association is sometimes delayed until the metaphase plate, the variability in the number of microchromosomes counted will be explained without accepting numerical changes in the karyotype. The presence of about 60–70 microchromosomes is diagnostic for the avian karyotype in contradistinction to that of Mammals where there is usually a more or less smooth gradation from the largest to the smallest chromosome. It is highly improbable that the avian karyotype came into being by chance without it having any evolutionary significance.

The problem of microchromosomes has not so far been subject to any study or discussion although it is of some importance in view of the several ways in which the microchromosomes are expected to behave differently from the so-called macrochromosomes. (*a*) Movements. Microchromosomes should move quicker and more easily than the long ones, whether it be during congression, orientation or distribution at the end of mitotic and meiotic prophase or during pairing and separation at the meiotic division. (*b*) Chiasmata. The microchromosomes are expected to have either one chiasma per bivalent or no chiasma at all. The possible absence of chiasmata would not lead to numerical inconstancy since the chromosomes are held together by the stainable substance accumulated at their ends. The absence or low frequency of chiasmata in microchromosomes is expected to have an effect on the linkage groups and particularly on their contents. (*c*) Structural alterations. Finally microchromosomes are expected to have prevailingly single chromosome breaks which would make intrachromosomal structural changes a very rare phenomenon among them. These three main properties of microchromosomes make the avian karyotype, genetics and evolution quite different from those of the mammals.

The avian karyotype, while retaining some large chromosomes and linkage groups, has found a different way of adaptation in the form of microchromosomes. That the ways of evolution of birds and of mammals are very different can be illustrated by two facts. *Phasianus* and *Meleagris*, two different genera, are karyologically indistinguishable—while in one species, *Gerbillus pyramidum*, there are three forms indistinguishable by taxonomic criteria but (Warhman & Zahavi, 1963) differing widely in their karyotype having diploid numbers of 40, 52 and 66 chromosomes.

SUMMARY

The chromosomes of the two parental forms and those of the hybrid do not show any detectable numerical or large structural differences. The pairing in the hybrid is frequently abnormal in that both free ends (highly charged with stainable substances) of many chromosomes show strong pairing, while midsegments repel each other. This partial failure of paring suggests that in the two parental forms gene mutations rather than chromosomal structural changes are responsible for incipient divergence.

It is suggested that the stainable substance of the chromosomes acts as a carrier mechanism ensuring orderly pairing, segregation and numerical constancy of

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bivalents in the hybrid where the partner chromosomes have become partly non-homologous.

The distinction between long and short chromosomes is typical for the avian karyotype, its evolutionary significance is discussed.

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