Microchromosomes in the American Red Fox, *Vulpes fulva*

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Introduction

Over the past three decades considerable variation in the number of chromosomes of the red fox, *Vulpes fulva*, has been reported, with diploid counts ranging from 34 to 42 (Table 1). Even discounting some of the earlier studies which employed less precise techniques than those used today, we still encounter reports of variations in chromosome numbers. Gustavsson and Sundt (1965) found different modal numbers for each of four red foxes from two different commercial fox farms; in an earlier study Gustavsson (1964) reported 38 chromosomes for the red fox. Moore and Elder (1965), on the other hand, failed to find any variation in chromosome number in the animals they studied and reported a diploid count of 38 chromosomes, although they noted that frequently a smaller number was found and attributed this discrepancy to the “loss” of one or more microchromosomes in the process of preparation.

In a recent paper reporting the presence of what he terms supernumary chromosomes in the harvest mouse, *Reithrodontomy megalotis*, Shellhammer (1969) has stated that accessory chromosomes have been reported in commercially raised foxes but that they have not been observed in the wild state.

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>Authors</th>
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<tbody>
<tr>
<td>42</td>
<td>Wodsedalek (1931)</td>
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<tr>
<td>34</td>
<td>Andres (1938)</td>
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<tr>
<td>34</td>
<td>Wipf and Shackelford (1942)</td>
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<tr>
<td>38</td>
<td>Makino (1947)</td>
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<tr>
<td>34</td>
<td>Wipf and Shackelford (1949)</td>
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<tr>
<td>38</td>
<td>Lande (1958)</td>
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<td>38</td>
<td>Gustavsson (1964)</td>
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<td>35, 36 and 37</td>
<td>Gustavsson and Sundt (1965)</td>
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<td>38</td>
<td>Moore and Elder (1965)</td>
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<td>35, 36 and 37</td>
<td>Gustavsson and Sundt (1967)</td>
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<td>37, 38, 39 and 40</td>
<td>Sasaki <em>et al.</em> (1968)</td>
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However, Sasaki et al. (1968), and more recently Makino (1969), in an abstract of the same study, have shown that microchromosome polymorphism does occur in both wild and domesticated foxes in Japan. The purpose of this paper will be to describe the results of our study of the chromosomes of the red fox, *Vulpes fulva*, in the United States.

**Materials and methods**

Two males and three females of the wild red fox, *Vulpes fulva*, and one female red fox from a commercial fox farm, were used in this study. The wild foxes were taken in the vicinity of Hanover, New Hampshire, USA; the domesticated fox was obtained from a commercial fox farm in Land O'Lakes, Wisconsin. All animals were adults except a cub which weighed 850 grams and is designated female number four in Table 2.

Chromosome preparations were obtained by short term leucocyte culture, according to the method of Moorhead *et al.* (1960); from explant cultures as described by Basrur *et al.* (1963); from bone marrow cells using a technique previously described (Benirschke *et al.* 1962); and/or kidney cell cultures using a modified procedure originally described by Rappaport (1956). All cells were fixed in glacial acetic acid and methyl alcohol (1:3); slide preparations were then air- or flame-dried and stained either with aceto-orcein or carbol fuchsin.

Since it had been suggested that the variable number of chromosomes in the fox might be caused by a loss of microchromosomes during slide preparation and was, therefore, artifactual and due to cell rupture (Gustavsson 1964, Moore and Elder 1965), cell cultures were established in large Leighton tubes containing standard microscope slides. At a time when the cells were rapidly dividing, colchicine was added to the cultures to accumulate metaphase...
figures; subsequently, the monolayers were treated with hypotonic saline and then fixed by flooding the culture tubes with fixative. In addition to preserving the integrity of the cells, it was hoped that this technique would allow us to determine the possible association of the microchromosomes with the larger chromosome of the complement and/or with each other.

Chromosomes were studied autoradiographically according to method previously described (Low and Benirschke 1966). Exposure times varied from 7-14 days. In essence, 6 hours prior to interruption cultures were exposed to 3H-thymidine. Following fixation and staining, chromosome spreads were photographed. After exposure suitably labeled mitoses were rephotographed and karyotypes were prepared of labeled and unlabeled chromosomes.

**Results**

Other investigators have generally divided the chromosomes of the fox into two groups, the larger group consisting of 16 pairs with medially or
submedially located centromeres (arranged karyotypically in descending order of size), and a smaller group of microchromosomes of variable number which, in the male, includes the Y chromosome. The X chromosomes were described as medium size submetacentrics.

Although admittedly somewhat arbitrary, since there is a nearly continuous transition from metacentric to submetacentric, for purposes of this study, and with the hope of detecting chromatid deletions, we have arranged the chromosomes into three groups as shown in Fig. 1. In descending order of size within each of these groups, the first consists of 7 pairs of metacentrics and the second is composed of 10 pairs of submetacentrics and the X. The third group, when present, is made up of a variable number of relatively small elements including the Y chromosome. With the possible exception of the Y, which in excellent preparations appears to have a submedially located centromere, the morphology of this group is difficult to ascertain. In some spreads these elements appear to have a terminal or subterminal centromere while in others the arms, although closely aligned and invariably paired, show no obvious connecting structure (Fig. 1). Although we have paired the microchromosomes in those karyotypes in which there are more than one of these elements, we do not know at this time whether they are homologues. Moreover, since in this study we have analyzed many metaphase spreads having an odd number of microchromosomes it is perhaps reasonable to assume that they are not diploid sets.

The results of chromosome counts on the animals studied are summarized in Table 2. As in the recent report by Sasaki et al. (1968) this analysis indicates that the variation in the number of microchromosomes in different cells of the same and in different foxes gives rise to the differences in numbers recorded. Contrary to their findings, however, which suggested to them a diploid number of 38 for the fox, a figure which is not consistent with the model number of 37 shown in their Table, the modal number which we obtained for the six animals studied was 35. If, on the other hand, we look at the chromosome counts for each animal studied, we find modal numbers of 35, 36, 37, and 38. In all six animals, however, the modal number of the macrochromosomes was constant: in the females, 34 including the X chromosomes; in the males 33 including the X chromosome plus the Y.

Animals 1 and 2 (♂) showed a modal chromosome number of 38, XY, 4 m (microchromosomes). While kidney cells of female number 1 give a modal number of 36, XX, 3 m, a significant number of these cells gave chromosome counts of 37 and 38. And although 36, XX, 3 m was the modal number of chromosomes in kidney cells of female number 2, leucocyte cultures from this animal yielded counts of 37, 38 and 40. Similarly, kidney and skin counts in female number 3 were 35, XX, 1 m; however, blood cells of this animal gave bimodal numbers of 36 and 37, XX, 2 m. Female number
four, the cub, was noteworthy in that cells of the kidney, skin, bone marrow and spleen all gave modal counts of 35, XX, 1m, with few analyzed cells having more or less than one microchromosome. In the adult animals, however, the number of microchromosomes ranged from none to seven. In the two male foxes these elements ranged in numbers from 3 to 7 with a modal number of 5. It must be remembered, however, that included in these
counts is the Y chromosome; hence, the macrochromosome counts for the males will be one less than the macrochromosome counts for the females studied because the Y is included with the microchromosomes.

Skin cells from female number 3 were grown and fixed directly on microscope slides as previously described. This was done in order to determine whether the usual air-dry method of making slide preparations might account for the variation in chromosome counts by causing cell rupture with the resulting loss of microchromosomes as suggested by others (Gustavsson and Sundt 1965, Moore and Elder 1965). Analyses of spreads prepared in this manner failed to reveal any apparent difference in the number or arrangement of the chromosome complement; consistent association of the minute elements with macrochromosomes or with each other, however, was not observed. Nevertheless, it was noted in some of the cells photographed that small pieces of chromatin material seemed to be breaking off the ends of the arms of some of the macrochromosomes (Figs. 2, 3, 4 and 5). In other chromosomes, pieces of equal size were detaching from the right and left arms, while in others only one arm appeared to contribute a fragment. Occasionally we observed a fragment that was completely detached while a second fragment appeared to be still in the process of separation having a strand of lightly staining material still connecting it to the macrochromosome. In other spreads some chromosomes had prominent terminal secondary constrictions which one might speculate could readily break off thereby giving rise to chromatin fragments (Figs. 6 and 7).

In an attempt to determine if the so-called microchromosomes might
arise as fragments from specific macrochromosomes, karyotypes from spreads with no microchromosomes were compared visually with cells with different numbers of fragments; it was hoped that in this manner it might be possible to observe more readily differences in the morphology of the chromosomes in spreads with and without fragments. Figures 2-5 show small pieces of chromosomes detaching from macrochromosomes; in many instances the fragments have the appearance of microchromosomes. Although we occasionally noted dimorphism in one of a pair of autosomes, we were unable, by inspection, to determine whether certain macrochromosomes were more likely to yield fragments; nor were we able to demonstrate consistently an obvious difference in the structure of any of the macrochromosomes, irrespective of whether or not the chromosome complement contained microchromosomes. The microchromosomes are relatively small, however, and if broken from the macrochromosomes they could be undetectable by inspection, particularly if the fragments break from the larger chromosomes of the complement in a non-random fashion.

We attempted autoradiographic studies of the chromosomes of female fox number 3 but insufficient labeling was obtained. Subsequently, female number 4 was successfully labeled and numerous spreads yielded informative patterns. As shown in Figs. 8 and 9 one of a pair of medium size sub-metacentric chromosomes was late labeling and is presumably the allocyclic...
X chromosome. Unfortunately, for the purpose of this portion of our study, the cells of this animal consistently contained only one microchromosome. Nevertheless, in all of the autoradiographs analyzed this element was nearly always heavily labeled.

**Discussion**

We have studied the chromosomes of five red foxes taken in the wild and one obtained from a commercial fox farm. As previously noted by Gustavsson and Sundt in the silver fox in Sweden (1965), and recently by Sasaki et al. (1968) in wild and domestrcated foxes in Japan, we also have observed a variable number of chromosomes in the cells of these animals caused by the presence of minute elements referred to as microchromosomes.

While several mechanisms may be operative in the production of this polymorphism, including the artifactual loss of one or more of these small elements from spreads as a result of cell rupture at the time of slide preparation (Gustavsson and Sundt 1965, Moore and Elder 1965), the results of this study suggest that this may not be the cause of the variation in cell count. That the poly-

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Fig. 9. Autoradiographs of female *Vulpus fulva*, number four (Table 2).
morphism is generally not due to chromosome loss resulting from cell manipulation, at least in this study, seems reasonably established by the method we employed to keep the cells intact; that is, by fixing the cell cultures as monolayers. In none of the cultures prepared in this manner did we find evidence of cell rupture; moreover, the infrequency of metaphase figures at variance with the established modal number of macrochromosomes further substantiates the observation that the integrity of these cells was maintained. Even in preparations handled in the usual manner, in which dispersed cells are fixed and air- or flame-dried on slides, little variation in chromosome number from the method referred to above was recorded.

Numerous photographed spreads did demonstrate what we interpreted as fragmentation of the macrochromosomes, a phenomenon which was seen in cultured and non-cultured cell preparations and whether or not the cultures had been inoculated with tritiated thymidine. The fragmentation was most often characterized by the apparent breaking off of the distal ends of the macrochromosomes. Although it is not possible to determine with certainty the origin of the microchromosomes, it is tempting to speculate that this may be the source of these small elements. In some preparations rather conspicuous telomeres were seen and it may be these structures which, in the absence of centromeres, could account for the fact that the fragments, when they become detached from the arms of the donor chromosome, remain paired. It would be of interest to pursue this hypothesis further with electron microscopic studies.

Why the chromosomes of the fox should fragment so often in this manner is not known. Of interest in this regard, however, is the fact that of the animals we studied only the cells of the cub consistently contained only one microchromosome while the adults demonstrated a greater variation and number of these elements, not only between animals but in the cells of different tissues of the same animal.

Consistent with the suggestion of Gustavsson and Sundt (1967) that the microchromosomes are heterochromatic, the results of our preliminary autoradiographic study showed the single microchromosome of the cub to be late labeling. Whether or not, as further suggested by Gustavsson and Sundt (1967), the microchromosomes are genetically inert fragments that are being eliminated from the chromosome complement remains to be proven.

Because of the variation in the number of chromosomes reported for the fox by different investigators, it has not been possible to establish a consistent diploid number for this species. Thus Todd’s (1970) recent suggestion of a basic diploid count of 38 for pedigreed stock does not seem to be well founded. While a modal number of 38 has been established by some investigators, the results of this study and others makes it quite apparent that this number may vary, for both domesticated and wild foxes, not only in different individuals, but also between cells of different tissues from the
The number and apparent morphology of the macrochromosomes of wild and domesticated foxes in this study and others, however, has been notably constant at 32 autosomes and one pair of sex chromosomes. It may thus be reasonable to suggest a modified convention for describing chromosome counts for this species similar to that suggested by Sasaki et al. (1968). Thus, an animal having a modal number of 36 chromosomes would have a diploid count of $2n=34$ plus 2 microchromosomes, i.e., $2n=34+2m$.

Summary

Studies of the chromosomes of a domesticated and five wild red foxes, *Vulpes fulva*, have produced chromosome counts of 35, 36, 37 and 38. Karyotypic analyses revealed that variations in the number of microchromosomes produced this polymorphism; moreover, it is suggested that these microchromosomes may arise from fragmentation of the macrochromosomes. Because it has not been possible to establish a consistent chromosome count for this species, it is further suggested that $2n=34$ be used to describe the basic diploid count and that the number of microchromosomes present be added to this figure. Thus, a total chromosome count of 36 would, by the suggested system, be recorded as $2n=34+2m$.

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References


