NOTES AND COMMENTS

C-BAND POLYMORPHISM IN A JAPANESE LUNG
FLUKE *PARAGONIMUS OHIRAI*
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1. INTRODUCTION

PARAGONIMUS OHIRAI is a trematode parasite of the rat lung and is one of five species of lung fluke found in Japan. The natural first intermediate hosts of this fluke are various brackish water snails, the second is a brackish water crab, and the definitive host is the Norway rat (*Rattus norvegicus*). This fluke is distributed over the southern half of Japan with its northernmost site in the Chiba prefecture (Miyazaki, 1961). According to the monograph of Benazzi and Lentati (1976) on flatworm cytogenetics the only karyotype of *Paragonimus* that has been reported is that of *P. kellicotti* ($2n = 16$). Recently, one of us (Y.S.) found that *P. ohirai* had $2n = 22$ (Sakaguchi and Tada, 1976). During the course of this investigation, a unique heteromorphism was found in the short arm of one of the medium sized chromosomes. The present paper demonstrates that the chromosome polymorphism in question results from the terminal deletion of a constitutive heterochromatic cap on that short arm. This event, it is suggested, may have followed a prior pericentric inversion.

2. MATERIALS AND METHODS

We examined testes of *Paragonimus ohirai* 40–50 days after inoculating rats with metacercariae isolated from specimens of the crab *Sesarma dehaani* (the second intermediate host) collected from the Maruyama river at Kinosaki (Hyogo prefecture, Japan). Chromosome preparations were made using the air-drying technique of Imai *et al.* (1977), with a modified hypotonic solution (1 per cent trisodium citrate solution with 0.0025 per cent w/v colchicine). In addition to standard Giemsa staining for conventional karyotype analysis, a modified version of the BSG method of Sumner (1972) was used for C-band staining. The modified procedures were as follows. Chromosome preparations were treated directly with 5 per cent barium hydroxide ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$) for 3–5 minutes and then treated with 2SSC for 30 minutes, both treatments at 65°C. Finally the preparations were stained with 4 per cent Giemsa solution (Merck) in M/15 Sørensen's pH 6.8 buffer for 90 minutes.

3. RESULTS AND DISCUSSION

The conventional karyotype of *P. ohirai* ($2n = 22$) consists of one pair of large submetacentrics (No. 1), eight pairs of medium or small sized

submetacentrics (Nos. 2-9) and two pairs of small metacentrics (Nos. 10-11) (fig. 1a). In this figure the size of the short arms of chromosome 4 is different in the two homologues (fig. 1a, arrow). This type of heteromorphism can be observed more clearly in meiotic bivalents at diakinesis (fig. 1b, arrow). C-band analyses of diplotene chromosomes revealed that one member of chromosome 4 has a heterochromatic cap at the tip of the otherwise euchromatic short arm (fig. 1c, arrow). Additionally both homologues have a large heterochromatic block at the proximal region of their long arms, whereas other chromosomes have only a small block of pericentromeric heterochromatin.

We denote chromosomes with the heterochromatic cap as B type and those having only a euchromatic short arm as C type (fig. 2). Besides these two types, two further variants, denoted here as B' and D, were also seen. In the former (B') the heterochromatic cap has been deleted from the short arm of the B type chromosome to form a small acentric fragment (fig. 1d, arrow). Variant (D) has a large heterochromatic block in the proximal region of the long arm which is about twice that found in the B type (figs. 2 and 3). Four types of cell, B/B, B/B', B/C and C/D, have been identified (table 1). Of the eleven individuals examined, three were B/B, four were B/C and the remainder were mosaics for B/B and B/B' or B/C and C/D combinations (table 1). As B/B' and C/D cells were infrequent they were presumably derived secondarily from B/B or B/C stem cells. If so, the Kinosaki population basically includes two chromosomally different individuals, namely B/B and B/C (fig. 1 insert).

We assume that the progenitor of chromosome 4 had a totally heterochromatic short arm (the A type chromosome in fig. 2). If a pericentric inversion were to occur in this chromosome it would produce a heterochromatic cap at the tip of the short arm as observed in the B type chromosome (fig. 2). As all individuals examined have either a B type or one of its derivatives (B', C or D type), the B type is presumably the basic

FIG. 2.—Schematic representation of a plausible evolutionary pathway describing chromosome 4 alterations in *P. ohirai*. Solid lines indicate pericentric inversion p.i. (AM) (A → B) and deletion of terminal heterochromatic cap (B → B' → C). Broken line indicates duplication of the heterochromatic block at the proximal part of the long arm. For details see text.

Plate I

FIG. 1.—Chromosome polymorphism in the Kinosaki population of *P. ohirai*. (a) The conventional karyotype; the arrow indicates a heteromorphic pair 4 while the insert includes the two major chromosome types (B/B and B/C) of C-band variants found in chromosome 4. (b) diakinesis; the arrow indicates a heteromorphic bivalent 4. (c) C-band stained diplotene bivalents; the arrow indicates heterozygosity for a terminal heterochromatic cap at the tip of the otherwise euchromatic short arm in bivalent 4. (d) C-band stained mitotic metaphase chromosomes; the arrow indicates a heterochromatic cap which has been deleted from the short arm of chromosome 4. The bar scales in this and fig. 3 represent 5 μ m.

FIG. 3.—The C/D type bivalent 4 at pachytene (i) and diplotene (ii and iii) of meiosis.

TABLE 1
C-band polymorphism of chromosome 4 in P. ohirai from Kinohsaki

Cell type*	Individual										
	1	2	3	4	5	6	7	8	9	10	11
B/B	43	37	56	41	49						
B/B'				3	2						
B/C					1	34	34	42	21	12	50
C/D						4	6				
Number of cells observed	43	37	56	44	52	38	40	42	21	12	50

* For details see fig. 2.

type in the Kinohsaki population. The next step is the induction of a C type chromosome by the loss of the heterochromatic cap of the B type (fig. 2). The mosaic individuals having B/B and B/B' cells indicate that the transformation of the B to the C type via B' is still occurring, and since five cells from two flukes were B/B' it suggests that the elimination of the heterochromatic cap occurs repeatedly in *P. ohirai*. The D type chromosomes found in B/C individuals presumably stem from the tandem duplication of the heterochromatic block of the long arm of the B type chromosome (fig. 2). We stress that the series of chromosome alterations $A \rightarrow B \rightarrow B' \rightarrow C$ proceeds irreversibly.

We have previously argued that pericentric inversions changing acrocentrics (A) to metacentrics (M) *i.e.*, ($A \rightarrow M$; termed as p.i. (AM)) occur statistically more frequently in karyotype evolution than the reverse ($M \rightarrow A$) (Imai and Maruyama, 1978), and that p.i. (AM) is one of the major rearrangements in mammalian karyotype evolution (Imai, 1978; Imai and Crozier, 1980). If this is so then metacentrics induced by p.i. (AM) should always have a heterochromatic cap since short arms of acrocentrics are mostly heterochromatic so that one of the two breakages inducing p.i. (AM) would be expected to occur in a heterochromatic short arm. Although chromosomes with heterochromatic caps are known in some mammals (*e.g.*, Bostock and Christie, 1975), such chromosomes are relatively infrequent and most of them appear to be related to the presence of a nuclear organiser region (*e.g.*, Goodpasture and Bloom, 1975). To account for this discrepancy between theoretical expectation and observation, Imai *et al.* (1977) proposed that such heterochromatic caps would be rapidly eliminated by breakage at the junction between the euchromatin and the heterochromatin. The B' type chromosome found in *P. ohirai* (figs. 1d and 2) may be the first substantive evidence supporting this assumption.

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