Continuous variation in Y-chromosome structure of *Rumex acetosa*

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The dioecious angiosperm *Rumex acetosa* has an XX/XY_1Y_2 sex-chromosome system. Each Y-chromosome is heterochromatic except for a minute terminal euchromatic pairing segment. The Ys are constant in size but have a variable centromere position. The centromeres can be located anywhere within the central 40 per cent of the chromosome but are excluded from the two distal 30 per cent regions. In a sample of 270 males from 18 different populations 68 distinct variants have been identified on the basis of Y-morphology. All populations are highly polymorphic with a minimum of four variants in a sample of ten males. The origin and significance of this massive variability is considered in this paper. Increased mutation rate of the Ys may be implicated in maintenance of this variation.

INTRODUCTION

Sex-determination in animals is usually genic and frequently associated with visibly-differentiated sex-chromosomes. Sex expression in plants, however, is usually more plastic, and is subject to environmental influences such as temperature and photoperiod (Heslop-Harrison, 1957). Additionally, even strictly dioecious plants seldom have visibly-differentiated sex-chromosomes and considerable controversy exists concerning the particular chromosomes involved in sex-determination in species as diverse as the gymnosperm *Ginkgo biloba* and the angiosperm *Spinacia oleracea* (Lewis and John, 1968).

Highly-differentiated sex-chromosomes have been found in a few plant species such as *Melandrium rubrum* and *Rumex acetosa*. In *M. rubrum* a pair of X-chromosomes is found in pistillate (female) plants, a single X and a larger Y in the staminate (male) plants. Detailed studies of this active-Y system have been carried out by Westergaard (1958). The subgenus to which *Rumex acetosa* belongs is characterised by an aneuploid sex-chromosome system, XX in females and XY_1Y_2 in males (Kihara and Ono, 1925). Several studies have shown this to be a dosage compensation system analogous to that in *Drosophila* (Ono, 1935). Although the heterochromatic nature of

these “inert” Ys has been described (Vana, 1972) variation in their structure has been overlooked.

Extensive heterochromatic content is a characteristic of many Y- and W-chromosomes. Indeed, some have argued that the process of heterochromatinisation itself was implicated in the initial phase of sex-chromosome differentiation (Jones, 1984). Highly-heterochromatic sex-chromosomes exhibit hypervariability in many groups of organisms and this is normally quantitative in character (Hamerton, 1971; Mengden 1981; Vorontsov *et al.*, 1980). In the human Y, for example, the long arm varies more or less continuously with respect to its C-band content while a single population of the rodent *Nesokia indica* contained three distinct Y-morphs in equilibrium proportions (Rao *et al.*, 1983).

Structural variation in sex-chromosomes independent of C-band content is unusual, but a remarkable case has been described in the W-chromosomes of the gekkonid lizard *Gehyra purpurascens*. Six W-morphs which differ by para- and pericentric inversions have been identified in a sample of only 30 females from 13 populations (Moritz, 1984). In *Rumex acetosa* we have found that the two Y-chromosomes, which are almost completely heterochromatic, show massive structural but not quantitative variability. Centromere location in each Y varies more or less continuously

such that in 270 males 68 recognisably-distinct complements have been found. The nature of this distinctive sex-chromosome system is reported in this paper.

MATERIALS AND METHODS

The plants used in this study were grown from seed collected in 18 natural populations of *R. acetosa*. In three populations—Navestock Heath, Essex, Torver, Cumbria and Hendra, Cornwall—seeds were collected from 40 females. In the other 15 populations, scattered widely through Britain, 10 seed samples were obtained. From each female parent a single male offspring was examined giving a total of 270 males. The precise locations of these populations will be given in a later paper.

For chromosome studies roots were pretreated with 0.05 per cent colchicine for 2 hours, fixed in 1:3 acetic alcohol and stained by the Feulgen method. Meiosis was examined in PMCs fixed in Carnoy and stained in 2 per cent acetocarmine.

RESULTS

The Karyotype

Pistillate (female) plants of *Rumex acetosa* have $2n = 14$ chromosomes comprising six pairs of autosomes and a pair of X-chromosomes. The staminate (male) plants carry a single X-chromosome and two distinguishable Y-chromosomes, designated Y_1 and Y_2 , giving a somatic number of $2n = 15$.

The six pairs of autosomes are individually recognisable on the basis of length, arm-ratio and secondary constrictions (figs. 1–2). Chromosomes

1 and 6 sometimes carry heterochromatic segments terminal on their short arms.

The X-chromosome is metacentric. It is the largest member of the complement ($7\ \mu\text{m}$), nearly twice the length of chromosome 1. During mitotic prophase the X-chromosomes and autosomes coil regularly, displaying no conspicuous blocks of heterochromatin. The two X-chromosomes in females have identical coiling patterns.

The Y_1 - and Y_2 -chromosomes, by contrast, are highly heterochromatic. They are distinguishable from the rest of the complement on the basis of coiling and staining at all stages of the mitotic cycle except metaphase and anaphase. In interphase nuclei, one large or two smaller chromocentres are present. These may either appear as dense masses or as extended chromosome-like structures. Plants can be reliably sexed by observations of interphase nuclei. During mitotic prophase the Ys are uniformly dark-staining with no evidence of intercalary euchromatin. Euchromatin is present, however, and is limited to a very short terminal region on each Y-chromosome (fig. 3). These segments on Y_1 and Y_2 are probably the pairing segments, homologous with the distal regions of the X arms.

The Y-chromosomes are intermediate in size between the autosomes and the X-chromosome. The Y_1 is on average 83 per cent of the X-chromosome length at metaphase, while the Y_2 is 74 per cent of the X. Most frequently both Y-chromosomes are metacentric. The position of the centromeres, however, is remarkably variable and the pattern of this variation is described below.

Sex-trivalent orientation

Maintenance of a 1:1 sex ratio in *R. acetosa* depends on the segregation of both Y-chromosomes to the same pole at anaphase-I. This is

Figure 1a and b¹ Chromosome complements of *Rumex acetosa* (a) ♀, $2n = 14 + 1B$; X-chromosomes arrowed. (b) ♂, $2n = 15 + 2Bs$; X, Y_1 and Y_2 arrowed

¹Bar in all figures represents $10\ \mu\text{m}$.

Figure 2 Karyotype of *Rumex acetosa*, showing the extreme Y_1 and Y_2 variants. The Ys are drawn with the pairing segments uppermost

ensured by regular production of a linear sex-trivalent by two terminal chiasmata and its subsequent orientation in a convergent manner (fig. 4). The system is extremely accurate. In 238 metaphase-I cells six trivalents in linear orientation (fig. 5) and three bivalent + univalent configurations were found (3.8 per cent abnormal PMCs) and at anaphase-I only five PMCs out of 371 (1.3 per cent) were XY:Y. Failure of regular segregation should give numerical sex-chromosome variants in the progeny and in 1095 seed grown plants two XXY individuals were detected.

Sex-chromosome structural variants

(a) X-chromosome

The X-chromosome is very stable and few variants have been detected. Two females carrying single acrocentric Xs and a male with an acrocentric X, all of standard length, have been found, presumably resulting from pericentric inversion. In addition two different X/autosome interchanges have been detected: one female was heterozygous for an X/3 interchange while two females and a male from the same population carried an X/2 exchange.

(b) Y-chromosomes

In conventionally-stained preparations chromosome morphology is defined by centromere position alone. In the Y-chromosomes of *R. acetosa*, however, the euchromatic pairing segment defines one telomere so centromere position in acrocentrics can be given a polarity. In about 40 per cent of the males (115/270) both Y_1 and Y_2 were metacentric while in the rest at least one Y was acrocentric. Four major classes can be defined on the basis of centromere position: both Ys metacentric (*mm*), Y_1 or Y_2 acrocentric (*am* and *ma* respectively), both Y_1 and Y_2 acrocentric (*aa*). In addition Y-polarity enables two classes to be distinguished in *am* and *ma* and four classes in *aa*. We designate centric location nearer the pairing segment "+" and away "-" (see fig. 3). All nine classes have been found in this sample of 270 males.

Figure 3 Mitotic prophase in male *Rumex acetosa*. Note the heterochromatic nature of the Ys. The euchromatic pairing segments are arrowed

Changes in the apparent position of the centromeres could result from addition or deletion of chromosomal material. This can be excluded by comparison of chromosome length in meta- and acrocentric Y-chromosomes. For Y_1 the metacentrics average 82.2 per cent of X and acrocentrics 83.9 per cent, for Y_2 73.5 per cent and 74.7 per cent respectively. Rather than addition/deletion, rearrangements such as pericentric inversions are implicated in centric transposition.

Centromere position is defined by long-arm length as a proportion of total length. Thus a metacentric has an arm-ratio of 0.5 and a telocentric 1.0. The smallest deviation from metacentricity which can be reliably assigned a polarity with respect to the euchromatic segment gives an

Figures 4 and 5 Metaphase-I in PMCs of *Rumex acetosa*. (4) Sex-trivalent in convergent orientation. (5) Sex-trivalent in linear orientation.

arm-ratio of about 0.53. In the following analysis the numbers of different Y-chromosome arm-ratios have been established within populations and then summed between populations. Thus chromosomes with the same arm-ratio occur in several populations. In view of the heterogeneity of the observations, each of these occurrences has been assessed as if it were independent. The actual numbers of males have not been considered because of the large difference in sample sizes between populations (10 vs 40).

In the Y_1 -chromosomes arm-ratios from 0.67^+ to 0.66^- have been recorded (figs. 6, 7). There is a more or less continuous range in the positive direction with no clustering. In the negative direction the majority of observations fall between 0.55 and 0.57 (20/33). In Y_2 similarly centromere position ranges from 0.68^+ to 0.71^- (figs. 6, 7). In contrast to Y_1 , the centromere location in the negative direction is continuously distributed (0.55^- – 0.71^-) while in the positive direction a concentra-

tion occurs from 0.56 to 0.58 (16/29). The number of acrocentric types of Y_1 and Y_2 are about equal, 64 and 71 respectively. In Y_1 positive and negative locations are equally frequent but Y_2 shows a slight negative bias (42:29).

The total number of morphologically distinguishable variants in all 270 males from 18 populations was 68. In order to show whether Y_1 and Y_2 variation is independent the variants can be plotted on a simple cross-shaped diagram, one axis representing Y_1 , the other Y_2 and the crossing representing the *mm* type (fig. 8).

In general, Y_1 and Y_2 appear to vary independently of each other. Thus the four classes am^+ , am^- , ma^+ and ma^- contain roughly equal numbers of variants (9, 8, 9 and 13 respectively). An anomaly occurs, however, within the class *aa*. Twenty-eight morphologically-distinct variants have been found, of which 13 are a^-a^- and 7 a^+a^+ . The classes a^+a^- and a^-a^+ are uncommon with only four variants in each (fig. 8).

Figure 6 Comparison of variation in centromere position in Y_1 and Y_2 chromosomes. Arm-ratios where centromeres are closer to the pairing segment are denoted '+' and further away '-'.

Figure 7 The location of centromeres in 270 Y_1 and Y_2 chromosomes drawn from 18 natural populations. Variants were assessed in each population and all have been plotted. Variants with the same arm-ratio and polarity may or may not be identical. Chromosomes with arm-ratios less than 0.54 cannot be assigned a polarity and have been omitted.

Population structure

All three of the large populations are extremely heterogeneous in Y-chromosome composition. Seventeen of the 40 males in the Navestock Heath population, for example, were *mm* while the remainder carried one or two acrocentric Ys. Ten different acrocentric types could be defined by arm-ratio and polarity. Four variants were detected once, and the remainder only 2–5 times each. Similar patterns of population heterogeneity were found in the Hendra and Torver populations with seven and 10 acrocentric variants respectively; the maximum number of any one variant was four. Most of these variants are demonstrably unique to the population.

The extreme Y-variability also characterises all 15 small populations. In the 10-male samples drawn from these populations a minimum of four and a maximum of eight variants have been detected. Detailed analyses of population structure will be reported in a later paper.

DISCUSSION

The Y_1 - and Y_2 -chromosomes of *Rumex acetosa* show a remarkable pattern of structural variation. The location of the centromere in each Y is

extremely fluid, apparently moving along the central 40 per cent of the chromosome about 20 per cent each side of the median position. There seem to be some preferred locations for the centromere when non-median. In Y_1 a concentration occurs 5–8 per cent further away from the terminal euchromatic segment, while in Y_2 a peak occurs 6–8 per cent nearer the euchromatic segment. All other locations giving acrocentric Y-chromosomes are about equally frequent.

In each Y there is a strict limit to free centromere movement at about 20 per cent from the median in both directions. Centromeres are excluded from the two distal regions, each comprising 30 per cent of the chromosome. It might be argued that the distribution of centromere position is normal about the median so that chromosomes from the tails of the distribution would be rare and hence undetected in our sample of 270 males. If this were so, however, we would expect the majority of acrocentric Y-chromosomes to have arm ratios between 0.54 and 0.56 which is clearly not the case.

What then limits centric disposition in *Rumex acetosa*? A possible mechanism is suggested by a functionally-similar system in mantid species in which males contain an X_1X_2Y trivalent. Between-species differences in centromere location indicate

Figure 8 Arm-ratios of the 67 morphologically-distinguishable Y_1Y_2 variants found in the 270-mile sample, in which at least one Y was acrocentric. The *mm* variant is omitted.

coordination of the $X_1 - Y$ and $X_2 - Y$ intercentric distances, maintaining an effectively isosceles triangle at metaphase-I to ensure regular disjunction (White, 1973). It could perhaps be argued that regular disjunction of the XY_1Y_2 trivalent in *R. acetosa* is dependent on more or less median centric disposition in both Ys. However, disjunction in males carrying the most extreme acrocentric Ys in combination with metacentrics is entirely regular. If disjunction efficiency does limit centric disposition, then breakdown must be a sudden threshold event. It is also difficult to understand why centromere movement, particularly away from the pairing segment, should adversely affect orientation, especially so if both Y_1 and Y_2 are affected in concert, or at least in a stepwise progression.

The deficiency of a^-a^+ and a^+a^- classes may result from imbalance of the sex-trivalent due to

shifts of the centromeres in opposite directions from the median position. The difference between centromere positions in the two Ys, however, may be considerable even in *ma* and *am* morphs, up to 21 per cent in *ma^-*. This explanation then is inadequate to explain the deficiency of a^+a^- and a^-a^+ classes in our sample.

The Y-chromosomes of *R. acetosa* are uniformly heterochromatic in conventionally-stained preparations. The boundaries between the centromere-containing region and the distal regions, therefore, must fall within a continuous stretch of heterochromatin. A finer analysis of the heterochromatin has yet to be undertaken, but others have found uniformity using fluorochromes and C-banding (Vana, 1972; Leeman and Ruch, 1983).

Hypervariability of sex-chromosomes has been noted in groups as diverse as mammals and

reptiles, in systems with both male and female heterogamety (Moritz, 1984; Vorontsov *et al.*, 1980). Most of the variation encountered is quantitative. Structural variation, unaccompanied by changes in C-band quantity, is less usual. The most striking example is the W-chromosome of the gekkonid lizard *Gehyra purpurascens*, in thirteen populations of which six W-morphs have been identified (Moritz, 1984). These W-chromosomes are differentiated by peri- and paracentric inversions and a centric shift, and an evolutionary scheme has been proposed deriving them from the standard Z-chromosomes. Only two populations with more than three females have been studied, with six and eight individuals, and both were monomorphic. Only one population contained two morphs, thus the species appears polytypic with respect to the W-chromosome. Clearly, the highly variable sex-chromosome system of *Gehyra* is qualitatively different from that in *Rumex acetosa* reported here.

All populations of *R. acetosa* are polymorphic with respect to the Y-chromosomes. Centric location in the Ys is so fluid that very few individuals of any one variant can be found in a single population. The simplest mechanism to account for these extensive centric shifts is pericentric inversion requiring two breaks. Both para- and pericentric inversions are demonstrable in *Gehyra* by G-, C- and N-banding patterns. In *R. acetosa*, however, the homogeneous nature of the constitutive heterochromatin of the Ys precludes direct demonstration of pericentric inversion. Other mechanisms of centric transposition require three or more simultaneous breaks and such occurrences will be rarer than simple inversions. In *Gehyra*, for example, 9 inversions have been implicated in the generation of the W-chromosomes, but only a single 3-break event (Moritz, 1984). If centric shifts in *R. acetosa* do result from inversion, then it is also likely that the *mm* class is a heterogeneous assemblage and the extent of polymorphism is even greater than that recorded.

The centric shifts in the heterochromatic Y-chromosomes probably have no mechanical significance at meiosis since the segregation of the trivalent is as regular in the most acrocentric Ys as in the more frequent metacentrics. In addition, chiasma formation between the X and Ys is exclusively terminal involving only the minute euchromatic pairing segments. Inversions then cannot fulfil a role in isolating gene blocks (Darlington, 1958). Indeed it seems likely that the Ys carry few, if any, major transcribed loci since they remain tightly coiled throughout the cell cycle. The struc-

tural variation of the Y-chromosomes then may be neutral although a selective component may be implicated in defining the limits of centric movement. If Y-variation is neutral monomorphic populations might be expected as a result of random fixation of variants. None has been found in the 18 populations examined; indeed all are more or less equally variable with at least four variants in random samples of 10 males. This massive variation perhaps results from an enhanced Y-chromosome mutation rate compared with the rest of the genome.

Sex-chromosomes in many groups of organisms are rich in particular DNA families named Bkm sequences originally isolated from the W-chromosomes of snakes (Singh *et al.*, 1980). These Bkm sequences have some of the properties of transposable elements (Jones, 1984) and enhanced rates of chromosome mutation have recently been ascribed to transposable elements, as for example in maize (McClintock, 1978) and *Drosophila* (Kidwell *et al.*, 1977). It is not known whether Bkm or similar transposon-like sequences are present in enriched amounts on the Y-chromosomes of *R. acetosa*. If they do occur, however, it is tempting to speculate that they are located preferentially in the central 40 per cent of the Y-chromosomes through which the centromeres wander. Exclusion of the centromere from the two terminal regions then may reflect a lack of potential rearrangement sites due to the absence of transposon-like sequences. This proposal is testable by molecular techniques.

Acknowledgements A. S. Wilby is supported by an S.E.R.C. research studentship.

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