

Abnormal and variable sex ratios in population samples of ladybirds

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While most ladybird species are believed to show a conventional 1 : 1 sex ratio, population samples from five different species of ladybird have been found to show significant excesses of females. The species involved are *Anatis ocellata*, *Exochomus quadripustulatus*, *Chilocorus renipustulatus*, *C. bipustulatus* and *C. nigrinus*. All possess neo-XY sex chromosome systems. It is possible that the excesses of females reflect the recombination of segments present at the ends of the neo-XY sex bivalent. If the products of recombination are more lethal in males than females, differences in sex ratio will result. An alternative hypothesis involves an interaction between Y-linked factors and maternally inherited factors, possibly of a transposable element type. The maintenance of such excesses of females in several species, in different taxonomic groups within the Coccinellidae, and the presence of differences in different populations of one of the species, must reflect a selective advantage for these excesses in natural populations of these species.

KEY WORDS:—Ladybird – sex ratio – neo-XY – transposable elements.

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INTRODUCTION

Most students of population genetics, or evolution, assume that there are usually approximately equal numbers of males and females in populations of bisexual species. This, in turn, is a consequence of equal numbers conceived and born, or of eggs fertilised and laid. Large deviations from a 1 : 1 sex ratio have sometimes found, of course, such as those associated with parthenogenesis, but they are not common in non-parthenogenetic species. Sex ratio abnormalities have even been found in some strains of the most genetically studied organism, *Drosophila* (Sturtevant & Dobzhansky, 1936), but they are not normal for *Drosophila*, where a 1 : 1 ratio usually prevails.

Because a 1 : 1 sex ratio is generally expected, and usually found when checked, there has been little incentive to check the sex ratios actually found in populations of a wide range of organisms, or to determine whether they are always constant, or can change at different times during the life cycle or year.

During the course of a study of the chromosomes of ladybirds, in natural populations, we have discovered an interesting situation where the adult sex ratio of at least five species, though certainly not all ladybirds, frequently deviates from a 1:1 ratio, there usually being an excess of females. In one of these species, different populations have been found to possess different adult sex ratios, which may vary in different months. Differences were also found when larvae and pupae moult through to adults. It would appear that a roughly 1:1 ratio may be achieved in some populations at the time that breeding takes place, but we doubt whether this is achieved in all populations.

MATERIALS AND METHODS

The four British and one Indian species of ladybird found to show an excess of females in population samples are *Anatis ocellata* (the Eyed ladybird), *Exochomus quadripustulatus*, *Chilocorus renipustulatus*, *C. bipustulatus* and *C. nigritus*. Taxonomically *Anatis* has little in common with the other three species: *Anatis* belongs to the Sub-Family Coccinellinae, Tribe Coccinellini, while the remaining four species belong to the Sub-Family Chilocorinae, Tribe Chilocorini. Cytogenetically, however, all five species do have one important feature in common: they all have neo-XY sex chromosome mechanisms (Fig. 1). The possible significance of this will be raised in the discussion.

Very little work has been carried out on populations of these ladybirds previously and so some description of their ecology and biology is necessary before describing the results. They are all arboreal, feeding on the Aphids, Coccids, Psocids and Collembola found on trees. *Anatis* and *Exochomus* are usually associated with coniferous trees, but adults are highly mobile, and can sometimes

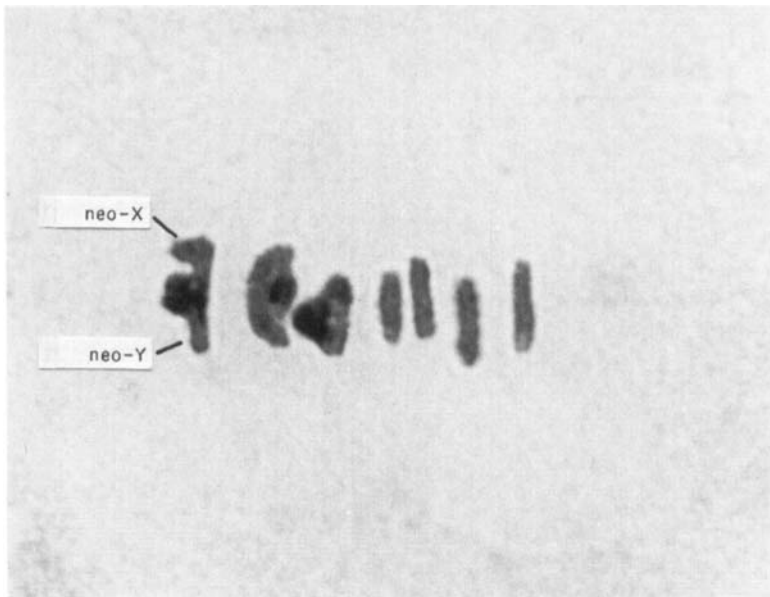


Figure 1. Metaphase I in *Exochomus quadripustulatus*, showing the neo-XY sex bivalent which accompanies the six autosomal bivalents.

be found on such deciduous trees as Maple and Sycamore, as well. Their larval stages are much more commonly restricted to coniferous trees. *Anatis* larvae are usually most common on Pine trees. Those of *Exochomus* are usually found feeding on the woolly aphids (*Adelges*) of Douglas Fir and Larch trees, but they too can also be found on Pine trees. On a world-wide basis, *Anatis* species are usually confined to conifers, but *Exochomus* is more variable, or adaptable, in its diet, being found on Citrus fruit trees in Turkey and Eucalyptus trees in Australia. *Chilocorus bipustulatus* is also sometimes found on coniferous trees, particularly pine trees, but usually in association with heather, on which it is more likely to breed. *C. renipustulatus*, is normally restricted to feeding on the Coccids found on Sallow or Willow. The *C. nigrinus* sent from India fed on Coccids attacking Citrus fruit trees.

All of the British species pass through only one generation per year. They overwinter as adults. *Anatis*, like most other ladybirds, hibernates and individuals may even bury themselves. *Exochomus* does not fully hibernate and may be seen on sunny winter days actively moving in search of food. In spring both species become increasingly active and, during March/April, can be seen mating on the branches and trunks of trees. *Anatis* lays its large yellow eggs singly, or in small clusters, on pine needles. They are laid conspicuously on the lower branches of large pine trees and on small pine trees in plantations. We do not know if they are also laid at the higher levels of tall pine trees. The smaller eggs of *Exochomus* are also laid singly, or in small clusters, in captivity, but, in the field invisibly in cryptic locations, most commonly on Douglas Firs.

A few weeks after egg laying adults start to die and/or fly away. Most are gone by the end of June and there is little overlap between old and new adults in the collections we have made from the lower branches of trees. *Anatis* eggs and larvae develop quickly and mature a full month before those of *Exochomus*. They pupate in June and all adults have emerged by early July. Soon after emergence they fly away to other trees, often to the tops of taller pine trees, and are not seen again on the branches on which the larvae and pupae were found.

Exochomus larvae develop slowly and the first few instars develop out of sight. Only the later instars can be found wandering freely on branches or needles, and collected by beating. Development can take up to four months in the cool climate of England. It is temperature-dependent and is quicker in the warmer climate of Turkey or in laboratory cultures (Uygun, 1978). Pupation begins in July and is completed during the beginning of August. Adults begin to emerge during July and most have emerged by the end of August. Few linger on until September. Newly-emerged adults do not show any interest in mating before the spring.

Chilocorus renipustulatus larvae pupate during the beginning of August. Adults begin to emerge by the middle of August. Emergence is fairly synchronized and is completed by the first week of September. Surprisingly, some of the newly emerged adults begin to show mating behaviour in September, a few weeks after emergence. It is unlikely that any eggs are laid before the winter snow and ice. Any sperm transferred in autumn is more likely to be stored in a spermatheca until the following spring.

None of these species are easy to collect in large numbers from different populations, on a regular basis. Although locally plentiful in good years, when found, populations are highly variable in numbers from site to site, year to year, month to month and even week to week. Thus, adults of *Anatis* and *Exochomus* fly

from tree to tree in search of food and are easily missed in collecting trips restricted to the lower branches of the most accessible trees. Sites found to possess many individuals one week may have little or nothing there a few weeks, months, or years later. The situation is further complicated by the growth of trees and the forestry practices found. Collections are easiest from the lower branches of young trees. Such trees grow quickly and their low branches are lopped off to produce knot-free, straight-grained wood. Many of the sites which were best when we started this work in 1984 are now almost useless. *C. renipustulatus* is not affected by forestry practices in the same way, but is affected by food distribution, and many trees may be examined before a small population is found.

Adults, larvae and pupae of the British species were collected during a three-year period (1984–1986) from populations in the southern half of Britain. At the start of the study, only hand collection was used, and this is the best method for collecting the larvae of *Anatis* and *C. renipustulatus* and the pupae of all species. This was soon supplemented by beating into large, wide-mouthed nets or onto white sheets. Most of the *Exochomus* collections of adults and larvae were carried out by beating. No differences in sex ratio were found in the two methods of collection, but beating clearly provides larger numbers and a more random sample. Larvae collected in the field were kept until they pupated. Some pupae were dissected, and the sex ratio of the pupae established, but most were allowed to moult through to adult, and the sex ratio of newly emerged adults compared with those collected in the field.

Collection is heavily dependent upon the weather: on warm, sunny days collection is easier than on cold wet days. Prolonged cold wet weather makes collection virtually impossible and probably also reduces numbers seriously due to effects on their food. Although the weather was acceptable, if not good, during 1984 and 1985, it was very cold and wet throughout 1986 and 1987, making collections more difficult, or impossible.

Many of the individuals collected were used for cytological studies or attempts to breed them in the laboratory. All individuals not needed for these purposes were returned to their original populations.

OBSERVATIONS

The most extensive and detailed observations and population sampling were carried out with *Exochomus* over a three-year period in an attempt to study of the frequency and distribution of supernumerary (B) chromosomes in natural populations. Sampling of the remaining species was more restricted, but, although the data from these species are smaller, they are fairly clear-cut. They will be described first, before listing the more detailed observations made with *Exochomus*.

Anatis ocellata

During the early summer (June) of 1985 about 100 last instar larvae of *Anatis* were collected from young pine trees at Lakenheath Warren in Norfolk. They were maintained in separate small plastic petri dishes to avoid cannibalism and fed on an artificial ladybird diet developed specially by the authors (Henderson & Albrecht, 1988) until they pupated. Sex determination in newly emerged young adults is easy in this species due to the abdominal differences found.

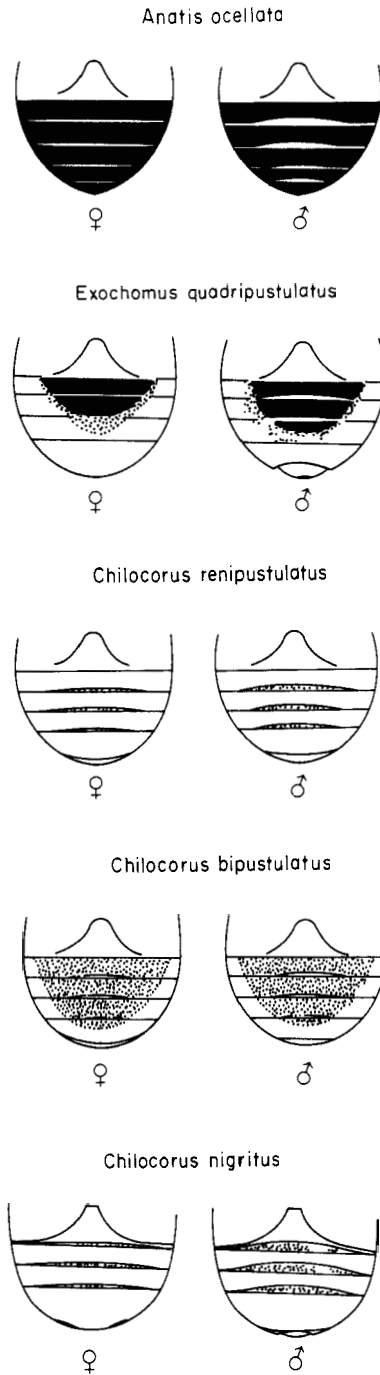


Figure 2. Morphological differences between males and females in the five ladybird species studied in this paper.

TABLE 1. The sex ratios of *Anatis ocellata* adults collected during 1985

Site	♀	%	♂	%	Totals
Lakenheath	12		7		19
Santon Downham	9		5		14
Kings Forest	2		0		2
Grimes Graves	2		2		4
Swaffham	5		3		8
Chicksands	7		4		11
Dene Park	1		1		2
Totals	38	63.3	22	36.7	60

Males are readily distinguished from females by the presence of broad ventral yellow-coloured flexure bands between the black segments (Fig. 2). These areas of thin cuticle enable the abdomen to be sharply incurvated during mating. Only narrow, less conspicuous bands are present in females.

In contrast to the situation in *C. renipustulatus* and *Exochomus* (see below) there was no evidence of either males or females emerging first. Of the 98 individuals which emerged as adults 68 (69.4%) were found to be female. This represents a highly significant departure from a 1 : 1 sex ratio ($\chi^2 = 14.73$, $P < 0.1\%$). It is, in fact, close to a 2 : 1 ratio.

No attempt was made to carry out widespread collections of *Anatis* adults during the following months, for the emphasis was on *Exochomus*, but during these collections small numbers of young adult *Anatis* were obtained from a number of sites. While the numbers of individuals collected from each location was generally small, a total of 60 adults was collected in all during the summer of 1985. Of these 38 (63.3%) were female (Table 1), again a statistically significant departure from a 1 : 1 ratio. The combined data, from both larval and adult collections made during the summer of 1985 are provided in Table 2. Of the 158 individuals collected, a total of 106 (67.1%) were female ($\chi^2 = 9.23$, $P < 0.5\%$). This very closely approaches the 66.6% of a 2 : 1 ratio.

While the data are limited and give no indication of any between-population differences of the type found in *Exochomus* (see below), they clearly indicate that the sex ratio of the newly emerged *Anatis* adults sampled was very far from a conventional 1 : 1.

TABLE 2. The sex ratios of all individuals of *Anatis ocellata* collected during 1985

Sample	♀	%	♂	%	Totals
Collected as larvae	68	69.4	30	30.6	98
Collected as adults	38	63.3	22	36.7	60
Total collected	106	67.1	52	32.9	158

TABLE 3. The sex ratios of *Chilocorus renipustulatus* individuals, collected as pupae or adults from different populations during 1984–1987

Year	Location	♀	%	♂	%	Totals
1984	Llanberis (N. Wales)	3		2		5
	Llanwrg (N. Wales)	18		9		27
	Kinsley (nr. Chester)	6		1		7
	Total 1984:	27	69.2	12	30.8	39
1985	Frimley (Surrey)	18	85.7	3	14.3	21
1986	N. of G. Yarmouth (Norfolk)	200	58.1	144	41.9	344
1987	ditto	81	53.3	71	46.7	152
Total collected		326	58.6	230	41.4	556

Chilocorus renipustulatus

Samples of *C. renipustulatus* were collected during 1984–1987 from widely separated geographic regions, ranging from N. Wales to Surrey and Norfolk (Table 3). External morphological differentiation between the sexes is slight in this species (Fig. 2), and, though possible, is not easy to make. Flexure bands are only slightly larger in males than females and both bands and abdomen are the same orange colour. All doubtful individuals were dissected to establish beyond doubt their true sex. In 1984 collections made by Dr. P. Kearns in N. Wales and N. England provided 39 adults of which 27 were found to be female (69.2%). In 1985 Dr. M. Majerus collected 21 adults from a population in Frimley (Surrey) of which 18 were females (85.7%).

In August 1986 a population of *C. renipustulatus* was discovered on the east coast of Norfolk, north of Great Yarmouth, at the time of pupation. A total of 344 pupae were collected from this region and returned to the laboratory to determine the sex ratio of the newly emerged adults. Over the next few weeks the percentages of males and females changed steadily, as shown in Fig. 3A. During the first week the numbers of males and females departed only slightly from 50% (52% female : 48% male). The percentage of females progressively increased in subsequent weeks, until, by the end of August, 66.7% of the emerging adults were female. Only six pupae remained in the first week of September and these all gave rise to females. Out of a total of 344 pupae, some 200 females were obtained, a percentage of 58.1%, which was a highly significant departure from a 1 : 1 sex ratio ($\chi^2 = 9.12$).

The same population was re-sampled on 15 August 1987. Because the summer was colder and wetter than the previous year, pupation and emergence occurred a week later. Collections were made in pouring rain and the leaves, branches and pupae were soaking wet. On return to the laboratory a comparable progressive increase in the percentages of emerging females was found from 16 August–7 September. This sample was collected at the peak of pupation before the emergence of any adults and the sex ratio of the first adults could be determined. By 17 August, 27 adults had emerged and the majority were males. Only 11 (40.7%) were females. A few days later the ratio had risen to 50% (15 out of 30).

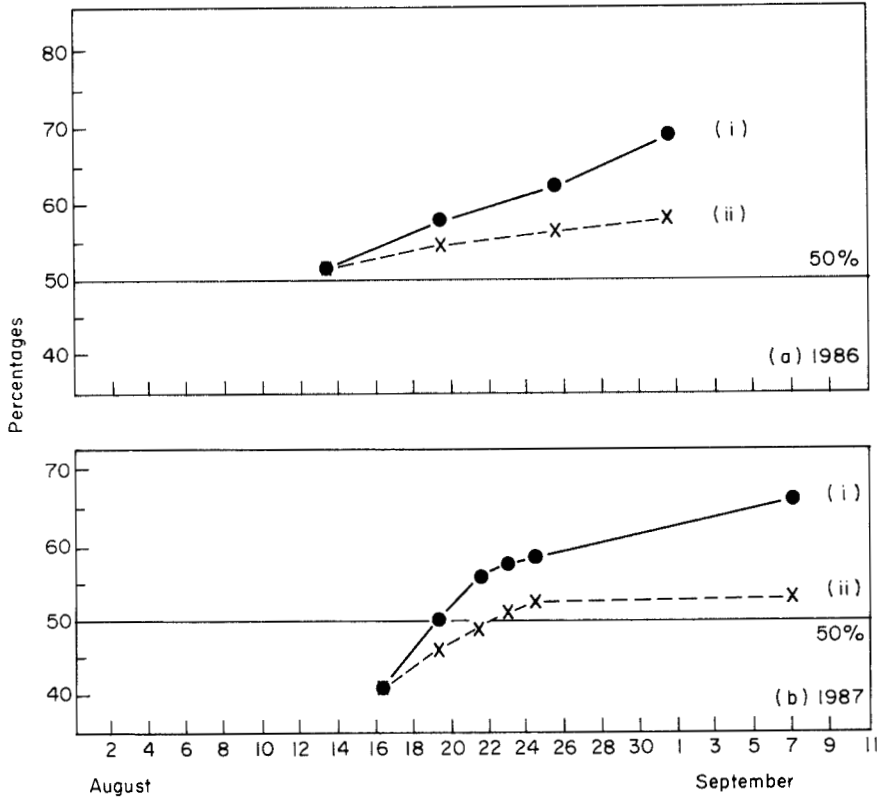


Figure 3. Variation in the sex ratio of adults newly emerged from pupae over time in *Chilocorus renipustulatus* during 1986 and 1987. (i) sequential totals, (ii) cumulative totals. Numbers of individuals contributing to each point are as follows, taken in order; (a) 1986 (i) 127, 113, 87, 27; (ii) 240, 317, 344. (b) 1987 (i) 27, 30, 32, 19, 41, 3; (ii) 57, 89, 108, 149, 152.

Thereafter the percentage of females steadily increased towards the 60% level. However, due to the wet collecting conditions, some of the wetted late emerging pupae and the willow leaves to which they were attached, became mouldy and died. Had they survived, the final percentage of females might have been higher and more comparable to that obtained the previous year. As a result, the final cumulative total obtained in 1987 was only 81 females from a total of 152 adults successfully emerged (53.3%). Despite this limitation the 1987 sample was valuable in revealing that (a) the initial sex ratio showed an excess of males as in *Exochomus* (see below) and (b) the non-cumulative totals showed a progressive increase to a final level of over 60% (Fig. 3B).

As in *Anatis*, therefore, there was an overall excess of females in this species, but the final percentage was lower than that found in *Anatis*. When all individuals collected over the four year period were pooled, the total was 556, of which the number of females was 326, giving an overall percentage of 58.6%. This highly significant departure from a 1 : 1 sex ratio is lower than the 66.6% of a full 2 : 1 ratio.

Chilocorus bipustulatus

Only a fairly small number of individuals of this species have been collected and most have been used for other purposes without recording their sex. The small number dissected, however, support the suggestion that the sex ratio here is similar to that of the other *Chilocorus spp.* In all a total of 33 individuals have been dissected, of which 20 were found to be female (60.6%).

Chilocorus nigritus

In October 1985 a sample of young adults of *C. nigritus* collected from Citrus trees at Jigani, near Banneraghata, India was kindly sent by post from the Commonwealth Institute of Biological Control, Bangalore, India. Identification of males and females proved to be possible on the basis of the larger abdominal flexure bands present in males (Fig. 2), but individuals were dissected to confirm identification. From a total of 63 individuals dissected, 48 proved to be females (76.2%), a massive departure from a 1 : 1 sex ratio.

Exochomus quadripustulatus

(a) *Between-population differences in newly emerged sex ratios:* These studies on ladybird sex ratios began in the summer of 1984 with a population survey of ladybird chromosomes. At the time it was not possible to sex ladybirds by external examination and it was noted that a disproportionately large number of females were found when individuals of *Exochomus* were dissected for cytological examination. The population from which most of these individuals were obtained was situated close to Old Warden, Bedfordshire. During June/July 1984 some 162 of these individuals were dissected and of these 105 (64.8%) were found to be female. During August a further 233 individuals were dissected, of which 145 (62.2%) were found to be female. Such wide departures from a 1 : 1 sex ratio were not found in other ladybirds of a conventional karyotype and an Xyp sex mechanism (see discussion).

Close examination of the abdominal segments of male and female ladybirds enabled one to identify the two sexes of most species with varying amounts of difficulty. *Exochomus* was not too difficult, there being a characteristic notch in the last abdominal segment of the males which was absent in females (Fig. 2). These criteria enabled one to determine the sex of collected individuals without dissecting them, and in some cases return them back to the wild to help maintain the populations.

In September a further 149 individuals were collected from Old Warden, of which 85 (57.0%) were now found to be female. There was thus a steady decline in the percentage of females collected from this site throughout the summer, from 64.8% in June/July to 57% in September (Fig. 4). The overall mean for this population during 1984 was 61.6% (Table 4) based on a total of 544 individuals.

Collections made from other populations in August and September yielded variable percentages (Table 4). At Chobham (Surrey) in September the percentage of females was only 52.4%. At Lakenheath in September the percentage of females was 54.7%, while collections from Santon Downham (near Brandon) gave 54.7% in August and 53.8% in September, with an overall mean of 54.2%. The differences found were not due to collecting techniques, nor the person collecting, because similar results were obtained with different collectors and techniques.

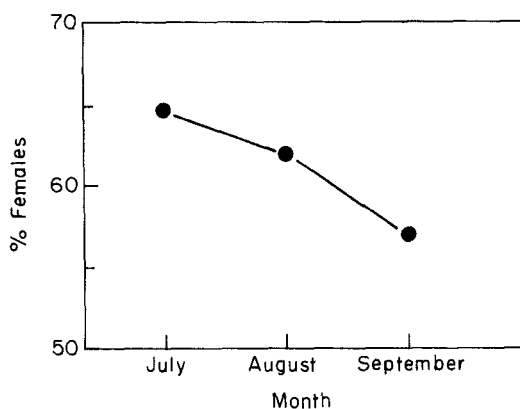


Figure 4. Decline in the sex ratio of females collected from Old Warden during the summer of 1984.

The results of the 1984 season's collections suggested that sex ratios in *Exochomus* populations were characterized by two features: (i) the presence of an excess of females in most populations and (ii) significant differences in different populations in the magnitude of this excess, which was not clearly related to geographic distribution.

In 1985 a major population survey was carried out which involved driving over the whole of the mainland Britain, to sample most forest areas from Cornwall to north of Scotland, including parts of north and south Wales. This revealed that *Exochomus* is not found in collectable numbers in Cornwall, Wales, Scotland nor the north of England. The main populations are restricted to a mid-band of the country stretching from the New Forest to Minehead and from Hereford to Peterborough (Fig. 5). Even in these populations large numbers are not easy to obtain on a collecting trip. The sex ratios obtained in the 1985 collecting season are provided in Table 5. It will be seen that in the vast majority of populations there was a significant excess of females. Once again, however, ratios varied from one population to another in a manner not clearly related to geographic

TABLE 4. The average sex ratios of *Exochomus* from all sites visited in 1984

Site	Visits	Totals collected			Nos. Females	% Females
		Adults	L + P	Overall		
Picket Hill Heath	1	3	—	3	3	100
Minehead	1	—	8	8	6	75.0
Grimes Graves	1	10	—	10	7	70.0
Quantock Hills	1	—	18	18	12	66.7
Thetford Forest	1	—	16	16	10	62.5
Old Warden	3	544	—	544	335	61.6
Brooks Green	2	30	—	30	18	60.0
Lakenheath	1	333	—	333	182	54.7
Santon Downham	2	118	—	118	64	54.2
Chobham	2	229	—	229	120	52.4
Kings Forest	1	1	—	1	—	0
Annual totals		1268	42	1310	757	$\bar{x} = 59.7$
Weighted percentage from annual totals						57.8



Figure 5. Geographic distribution of the sites listed in Table 7 over the southern half of England.

TABLE 5. The average sex ratios of *Exochomus* from all sites visited in 1985

Site	Visits	Totals collected			Nos. Females	% Females
		Adults	L+P	Overall		
Warren	1	35	—	35	30	85.7
Chicksands	1	11	2	13	11	84.6
Shoreham	1	15	21	36	30	83.3
Brooks Green	2	12	31	43	35	81.4
Swaffham	3	62	—	62	47	75.8
Newbury	1	4	—	4	3	75.0
Byfleet	1	18	—	18	13	72.2
Hereford (Q)	1	2	11	13	9	69.2
Hereford (H)	1	20	29	49	33	67.3
France	1	5	64	69	44	63.8
Cadnam	1	30	—	30	19	63.3
Quantock Hills	1	19	—	19	12	63.2
Old Warden	4	245	17	262	150	57.3
Sandringham	2	59	29	88	50	56.8
Grimes Graves	2	41	19	60	34	56.7
West Tofts	1	50	—	50	28	56.0
Chobham	2	110	—	110	60	54.5
Santon Downham	3	207	13	220	113	51.4
Sandy	6	332	54	386	194	50.3
Mundford	1	12	—	12	6	50.0
Minchhead	1	2	—	2	1	50.0
Lakenheath	3	115	—	115	55	47.8
Peterborough	1	129	—	129	60	46.5
Kings Forest	3	93	—	93	42	45.2
Keel	1	6	—	6	2	33.3
Annual totals:		1634	290	1924	1081	$\bar{x} = 61.6$
Weighted percentage from annual totals:						56.1

TABLE 6. The average sex ratios of *Exochomus* from all sites visited in 1986

Site	Visits	Totals collected			Nos. Females	% Females
		Adults	L + P	Overall		
Old Warden	1	30	7	37	27	72.9
Eartham Wood	1	26	34	60	43	71.7
Kings Forest	1	8	5	13	9	69.2
Santon Downham	1	7	21	28	19	67.9
West Tofts	3	49	29	78	52	66.7
West Stow	2	67	29	96	63	65.6
Swaffham	1	35	12	47	30	63.8
Lowestoft	1	29	51	80	50	62.5
Sandringham	1	29	20	49	30	61.2
Coates	1	1	4	5	3	60.0
Peterborough	1	35	18	53	31	58.5
Grimes Graves	2	17	76	93	53	57.0
Portslade	1	4	—	4	2	50.0
Cadnam	1	2	—	2	1	50.0
Sandy	4	17	100	117	58	49.6
Lakenheath	1	20	7	27	12	44.4
Brooks Green	1	5	6	11	3	27.3
Annual totals:		381	419	800	486	\bar{x} = 58.7
Weighted percentage from annual totals:						60.7

distribution. A small number of samples actually showed less than 50% due to sampling.

The weather during the summer of 1985 was not particularly good, but the summer of 1986 was extremely cold and wet. Heavy rainfall made collecting difficult and reduced ladybird numbers appreciably, probably due to the shortage of Aphids and Coccids. The cold winter between 1985 and 1986 might also have reduced numbers surviving over winter. Collections made in 1986 were small and difficult to make. The figures obtained are shown in Table 6. Once again, between population variation was evident.

A summary of the three years' collections of *Exochomus* from all populations (pooling adults, larvae and pupae) is provided in Table 7. Annual totals and three-year totals are provided for cross-reference. The overall tendency for an excess of females is clearly illustrated from year to year. Because small samples can produce spurious variation in the percentages obtained, the annual variations in sex ratios shown by the largest and most fully sampled populations over this three year period are listed separately in Table 8. The overall weighted population percentages for the three-year period are provided in the final column. If variations in annual sex ratio were due to sampling error, these should cancel each other out and all populations approach equality. It is clear from Table 8 that this is far from the case. Although annual variations due to either ecological reasons or sampling error were found, it is clear that these populations differ from one another in a roughly repeatable, and hence presumably meaningful, fashion. Thus, the percentage of females at Old Warden was always close to 60%. At Swaffham it was usually higher, while at Lakenheath and Sandy it was closer to 50%. These observations are of particular interest because the populations at Old Warden and Sandy, usually differing by 10%, are geographically only a few miles apart.

TABLE 7. The final sex ratio data from 32 population samples of *Exochomus* collected over three years (adults, larvae and pupae pooled). (Hereford Q=Queens Wood, H=Haugh Wood; France=Paimpont and Neant)

Year	1984			1985			1986			3-Year totals		
	♀	T	%	♀	T	%	♀	T	%	♀	T	%
1. Sandringham	—	—	—	50	88	56.8	30	49	61.2	80	137	58.4
2. Swaffham	—	—	—	47	62	75.8	30	47	63.8	77	109	70.6
3. Mundford	—	—	—	6	12	50.0	—	—	—	6	12	50.0
4. West Tofts	—	—	—	28	50	56.0	52	78	66.7	80	128	62.5
5. Grimes Graves	7	10	70.0	34	60	56.7	53	93	57.0	94	163	57.7
6. Santon Downham	64	118	54.2	113	220	51.4	19	28	67.9	196	366	53.6
7. Thetford Forest	10	16	62.5	—	—	—	—	—	—	10	16	62.5
8. Lakenheath	182	333	54.7	55	115	47.8	12	27	44.4	249	475	52.4
9. Kings Forest	—	1	0	42	93	45.2	9	13	69.2	51	107	47.7
10. West Stow	—	—	—	—	—	—	63	96	65.6	63	96	65.6
11. Lowestoft	—	—	—	—	—	—	50	80	62.5	50	80	62.5
12. Peterborough	—	—	—	60	129	46.5	31	53	58.5	91	182	50.0
13. Sandy	—	—	—	194	386	50.3	58	117	49.6	252	503	50.1
14. Old Warden	335	544	61.6	150	262	57.3	27	37	72.9	512	843	60.7
15. Chicksands	—	—	—	11	13	84.6	—	—	—	11	13	84.6
16. Warren	—	—	—	30	35	85.7	—	—	—	30	35	85.7
17. Shoreham	—	—	—	30	36	83.3	—	—	—	30	36	83.3
18. Chobham	120	229	52.4	60	110	54.5	—	—	—	180	339	53.1
19. Byfleet	—	—	—	13	18	72.2	—	—	—	13	18	72.2
20. Brooks Green	18	30	60.0	35	43	81.4	3	11	27.3	56	84	66.6
21. Coates	—	—	—	—	—	—	3	5	60.0	3	5	60.0
22. Eartham Wood	—	—	—	—	—	—	43	60	71.7	43	60	71.7
23. Portslade	—	—	—	—	—	—	2	4	50.0	2	4	50.0
24. Picket H. Heath	3	3	100.0	—	—	—	—	—	—	3	3	100.0
25. Cadnam	—	—	—	19	30	63.3	1	2	50.0	20	32	62.5
26. Newbury	—	—	—	3	4	75.0	—	—	—	3	4	75.0
27. Minehead	6	8	75.0	1	2	50.0	—	—	—	7	10	70.0
28. Quantocks	12	18	66.7	12	19	63.2	—	—	—	24	37	64.8
29. Hereford (Q)	—	—	—	9	13	69.2	—	—	—	9	13	69.2
30. Hereford (H)	—	—	—	33	49	67.3	—	—	—	33	49	67.3
31. Keele	—	—	—	2	6	33.3	—	—	—	2	6	33.3
32. France	—	—	—	44	69	63.8	—	—	—	44	69	63.8
Annual totals	757	1310	59.7	1081	1924	61.6	486	800	58.7	2324	4304	57.9
Weighted %			57.8			59.6			60.7			

TABLE 8. Annual variations in sex ratio in the largest and most sampled populations of *Exochomus* over the three year period 1984–1986, with (weighted) overall population means

Site	Total	%	Total	%	Total	%	Total	%
	1984	Female	1985	Female	1986	Female	3 years	Female
Swaffham	—	—	62	75.8	47	63.8	109	70.6
Brooks Green	30	60.0	43	81.4	11	27.3	84	66.7
West Tofts	—	—	50	56.0	78	66.7	128	62.5
Old Warden	544	61.6	262	57.3	37	72.9	843	60.7
Sandringham	—	—	88	56.8	49	61.2	137	58.4
Grimes Graves	10	70.0	60	56.7	93	57.0	163	57.7
Santon Downham	118	54.2	220	51.4	28	67.9	366	53.6
Chobham	229	52.4	110	54.5	—	—	339	53.1
Lakenheath	333	54.7	115	47.8	27	44.4	475	52.4
Sandy	—	—	386	50.3	117	49.6	503	50.1
Peterborough	—	—	129	46.5	53	58.5	182	50.0

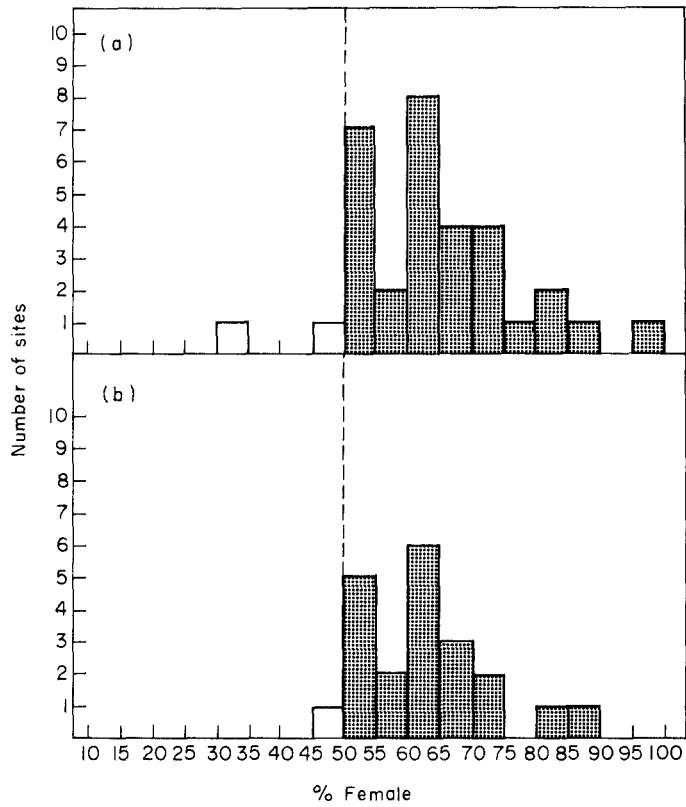


Figure 6. Distributions showing the numbers of sites with different percentages of females (a) all sites with more than 2 individuals collected, (b) all sites with more than 20 individuals collected.

Histograms showing the sex ratios at these sites are also provided for comparison (Fig. 6A, B). These are instructive in showing a tendency for bimodality in the sex ratios found. Peaks are shown close to 50% and over 60%, with very few populations actually showing values of between 55–60%, which are the final means obtained for these populations.

(b) *Differences in dates of emergence of males and females*: Sex ratio variants of the types observed above might conceivably have involved ecological differences in the behaviour of males and females affecting their ease of collection. To show that this was not likely to be a major factor contributing to the excesses of females usually found, larval and pupal collections were made, as in the other species described above. In most of the *Exochomus* populations from which larvae were collected, the relationship was found to be essentially similar to that described above for *C. renipustulatus*. Initially a small excess of males was followed by a progressive increase in the percentage of females until a value of over 60% was reached (Fig. 7A, B). Two of the population samples differed, for some reason. That from Sandy showed this initial increase in the percentage of females, followed by a final decrease; that from the Lowestoft area showed an erratic decrease in the percentage of females. In Fig. 7A the totals for all sites, excluding Sandy and Lowestoft, are provided, while in Fig. 7B all sites, including Sandy and Lowestoft, are pooled.

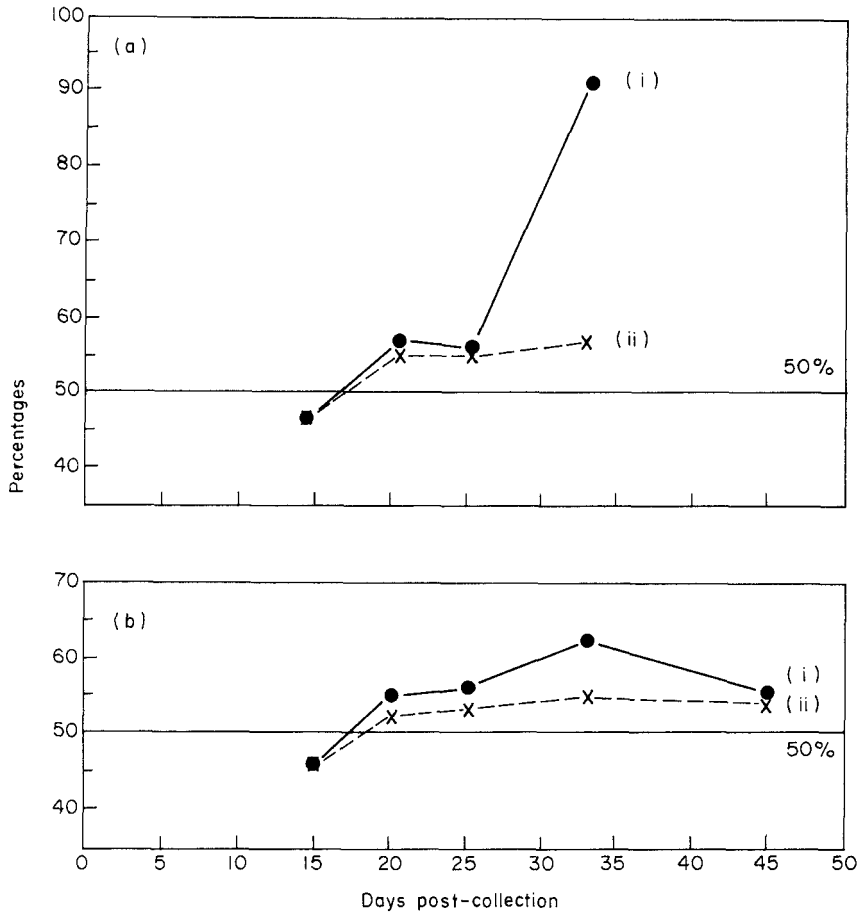


Figure 7. Variation in the sex ratio of adults newly emerged from collected pupae and pupated larvae over time in *Exochomus quadripustulatus* in 1986. (a) all sites excluding Sandy and Lowestoft. (b) all sites including Sandy and Lowestoft pooled. (i) sequential totals, (ii) cumulative totals. Numbers of individuals contributing to each point are as follows, taken in order: (a) (i) 45, 120, 77, 11; (ii) 165, 242, 253. (b) (i) 68, 160, 91, 78, 22; (ii) 228, 319, 397, 419.

Collection of adults from the field would involve sampling all adults which had emerged up to the date of collection, unless migration of newly emerged adults occurred. To illustrate what this would mean for the larval/pupal data, cumulative ratios are provided in Fig. 7A, B for comparison with the daily emergence data. The curves are flatter, with less variation, but broadly follow the non-cumulative curves.

One set of observations at variance with most of the larval and pupal data, with the exception of that from Lowestoft, is the reduction in the percentage of female adults found during the summer of 1984 at Old Warden (Fig. 4). We are unable to explain these reductions. Population differences may exist in warm sunny summers compared with cold wet summers either in the rate of emergence of females, or in their mortality or migration.

(c) *Size differences between males and females*: An incidental observation which is worth reporting as it has not been emphasized in the literature, is that small

differences in size exist between males and females in *Exochomus*. These are not sufficiently bimodal to be used for separating the two sexes. Distributions overlap extensively and large males and small females are quite common, but, on average, males are smaller than females. Linear measurements were not undertaken, but samples of about 100 individuals were weighed carefully and the individual weights determined for both newly emerged and overwintered adults. The results are as follows: Newly emerged (August 1985): ♂ = 0.0072 g, ♀ = 0.0086 g

Overwintered (March 1987): ♂ = 0.0067 g, ♀ = 0.0077 g

The smaller weights recorded in 1987 are likely to reflect both the poor food and climatic conditions during larval and adult development the previous summer and the cold winter experienced during 1986/1987.

(d) *Overwintered sex ratios*: All of the observations reported above concern the sex ratios of newly emerged young adults. These mature during the autumn, overwinter without complete hibernation, and mate during the following spring. It is difficult to determine the percentages of males and females in populations during winter and spring because individuals are even less collectable in large numbers during the cold wet winter weather. Limited collections have been carried out during the warmer days of winter and spring in some populations for comparison with the newly emerged summer data. Unfortunately collections could not be made from those populations which showed the greatest deviations from a 1:1 sex ratio, such as Brooks Green and Swaffham, because of their greater distance from Cambridge and the smaller size of their collectable populations. Those collections which were made were from Sandy, Lakenheath, Grimes Graves and Old Warden. Three of these populations do not show a great excess of females and would not be expected to change greatly during winter months.

Collections made during winter months gave the following figures: Sandy 52.8% (176 individuals); Lakenheath 55.4% (271 individuals); Grimes Graves 57.4% (94 individuals) and Old Warden 60.7% (28 individuals). These are comparable with the late summer means. Unusually low percentages were obtained during March/April from Lakenheath (43.3%; 543 individuals) and Grimes Graves (45.8%; 249 individuals), but this was followed during late April at Lakenheath by a more normal value of 57.1% (114 individuals). The data are not extensive, but are compatible with the suggestion that extensive mortality of females does not occur during the winter months to give a precise 1:1 sex ratio. The ratio probably remains at its autumn level until the following spring. The excess of males found during the two cold spring samples at Lakenheath and Grimes Graves probably reflect the greater mobility of males on these days. Due to their smaller size they may warm up and become more active on cold spring days. A few weeks later the percentage of females was found to be back to normal.

DISCUSSION

Although detailed studies of sex ratios of other species of ladybird were not carried out by ourselves during the course of this study, we understand that most species of ladybird are believed to exhibit conventional 1:1 sex ratios. For some years laboratory experiments have been carried out in this Genetics Department by Drs Majerus, O'Donald and Kearns on the topics of melanism and sexual

selection. Some of these have involved the 2-spot, 7-spot and 10-spot ladybirds. Although accurate sex ratios were not measured during these experiments, important variations from a 1:1 sex ratio were not considered to be found. Similarly, a small population sample of the 22-spot ladybird made in 1984 by the authors at the time of the *Exochomus* collections gave a normal 1:1 ratio (38 females:36 males).

It is equally clear, from the observations reported in this paper, that some ladybird species do depart from a conventional 1:1 ratio. They show an excess of females which may be variable and could be derived from an initial 2:1 ratio. Three main questions are raised by these observations and will be considered in turn in the discussion: (a) Why should some species differ from others in their sex ratios or, to put it another way, what is the underlying mechanism responsible for the differences found? (b) Why do these differences persist in these species, possibly for long periods of time, and why does selection not act to restore a 1:1 sex ratio? (c) Why do different populations, e.g. in *Exochomus*, differ from one another in their sex ratios?

(a) *Possible mechanisms for the sex ratio variation*

The answer to the first question is probably provided by the sex chromosome constitution of the species involved. Most of the common ladybird species possess a simple Xyp (parachute) sex chromosome system in which a large X chromosome is attached to a small Y chromosome at metaphase I of meiosis by a small persistent nucleolus (Smith, 1953; John & Lewis, 1960). This Xyp nucleolus can be stained with the more recent silver-staining techniques (Henderson, unpublished). Chiasma formation does not appear to be involved in their bivalent formation and co-orientation. Sex chromosome segregation will always produce normal separation of X and Y chromosomes and a 1:1 sex ratio would be expected from their reunion at fertilization.

All of the species found to possess abnormal sex ratios, with an excess of females, are also characterized by the possession of a changed sex chromosome system. They all have neo-XY systems in which the diminutive Y is lost and the large X has undergone X-autosome translocation, with the production of a characteristic heteromorphic bivalent at metaphase I (Fig. 8). The autosome, now firmly translocated to the X chromosome, will initially have a full complement of autosomal genes, which will be fully active genetically. If possible, and if required, selection could reduce the genetic activity of the neo-Y chromosome by the transfer of indispensable genetic functions from this chromosome to others within

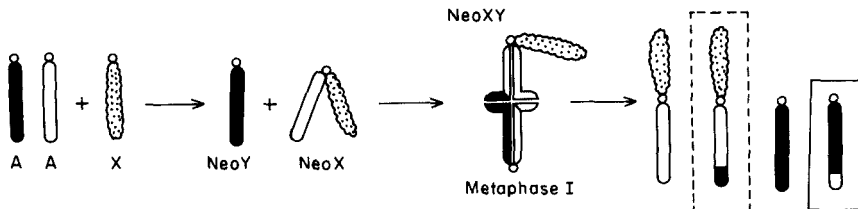


Figure 8. Diagrammatic representation of the evolution of a neo-XY sex chromosome system by X-autosome translocation and the consequences of chiasma formation. Complete lethality of the male recombinant alone would produce a 2:1 sex ratio. Variable lethality of the female recombinant also would give variable sex ratios.

the complement. This reduction in the genetic activity of the neo-Y can lead to its reduction in size ('erosion') and heterochromatinization.

Such changes take time, for they will depend upon the right changes occurring at the right time and the right place. In the meantime, one of what was originally an autosomal pair becomes restricted to one sex, the other is found in both sexes. This isolation could lead to genetic differences arising. The association of the neo-XY bivalent does not rely on a nucleolus, as in Xyp bivalent, but on a conventional chiasma between the originally autosomal partners, which can often be seen to be subterminal (Figs 1, 8).

The excess of females found in newly emerged adults cannot be due to positive action *increasing* the numbers of females, but must clearly reflect negative action, *reducing* the numbers of males in some way. In view of the correlation noted between the presence of an excess of newly emerged females and the presence of a neo-XY sex chromosome mechanism, it could be that the effect is a result of crossing-over between the autosomal elements of the neo-XY sex chromosome pairs. However, at least three different explanations are possible, conventional and unconventional, as to how crossing-over could bring about an excess of females: (i) The neo-XY sex chromosomes may have genes close to their ends which are lethal when separated by recombination, the degree of lethality differing in males and females. In the case of the males it may be a reflection of some form of gametic or zygotic lethal so that only one class of gamete or zygote gives rise to an adult. In females it may either be non-lethal or it may vary in its lethality in different species or populations (Fig. 8). If non-lethal a 2 : 1 sex ratio would result. If lethality was variable, ratios of between 1 : 1 and 2 : 1 would be found.

In order to determine whether the reduction in male numbers is due to a gametic or zygotic effect, the course of meiosis and sperm development in all affected species was examined and found to be normal. No defective spermatids are produced and no defective sperm were found. All sperm bundles are normal and all sperm motile when activated in saline. Any effect on male survival must therefore be due to effects on the early development of the zygote, or embryo, and not involve any form of abnormal sperm production.

For this explanation to work from one generation to the next, the extra, recombinant, X females ought either to die before the time of mating, or be sexually sterile. This is because the bringing together of a recombinant X and a Y would no longer produce the same result after crossing-over. Limited collections were attempted during winter and spring in an attempt to determine whether the ratio of females remains the same as that found in late summer. The data were not extensive but suggested that the ratios during winter and spring were probably close to those of the summer months and that extensive mortality of females, to establish a precise 1 : 1 sex ratio, probably does not take place. It is possible that the excess involves sterile females, which do not breed, but this would seem a wasteful explanation.

(ii) An alternative, and more speculative, suggestion would be that the segments separated by recombination do not bring about their effects due to the action of conventional Mendelian genes, but by other chromosomal elements, which may even be protein. Thus, the addition of a piece of maternal X chromosome to a Y may impair its function in early embryonic development, e.g. resulting in its non-disjunction. The addition of a Y segment to an X may have a

more variable effect, resulting in the differences found in different populations. If a female succeeds in developing and gives rise to an adult the recombinant X chromosome may become changed during developmental mitoses and 'feminised', to produce a completely maternal X once again. With this hypothesis, death or sterility of the excess females would not be necessary.

Although speculative, there is clear evidence that this type of behaviour can and does occur in some species. Thus, the elimination of chromosomes during the embryonic development of certain species of the Cecidomyidae is often extremely complex and incredible. Some of the patterns of behaviour found have been summarized by Lewis & John (1963). In some cases, such as that found in *Sciara coprophila*, for example, selective non-disjunction eliminates only paternal X chromosomes during development, not maternal. But those paternal X chromosomes were contributed to the male zygote by its mother, that is they were originally maternal before becoming 'masculinized' during the developmental mitoses of the male. Presumably chromosomal proteins or similar substances are changed during the development of both males and females so that sex chromosomes become recognisably different from one generation to the next. Such behaviour could possibly be involved here in ladybirds, in the behaviour of the neo-X, neo-Y and their products of recombination.

(iii) The third explanation involves the action of maternally inherited factors (or possibly transposable elements) of the type suggested to be involved in sex ratio variants of the rice weevil *Sitophilus oryzae* (Holloway, 1985). Most populations of this species show a 1:1 sex ratio, but some populations show a significant excess of females. It is important to note that this species is a beetle which has also come to possess a neo-XY sex chromosome system of the type found in the affected ladybird species. Detailed studies of the inheritance of this phenomenon in laboratory crosses of three different geographic strains led Holloway to conclude that the data supported a model involving the interaction of a non-chromosomal maternally inherited factor and a Y-linked factor. There was also some close resemblance to the phenomenon of hybrid dysgenesis (Kidwell, Kidwell & Sved, 1977). It is possible that different populations differ in the presence of neo-XY associated transposable elements which are capable of affecting sex ratio.

What evidence is there for the existence of transposable elements and hybrid dysgenesis in ladybirds? In a study of the phenomenon in *Drosophila* it was shown that the hybrid dysgenesis phenomenon is associated with extensive chromosome fragmentation in the relevant crosses (Henderson, Woodruff & Thompson, 1978). In the present material identical chromosome fragmentation was seen to occur spontaneously in a few cells of *Exochomus* individuals collected from Old Warden and Kings Forest, in otherwise normal testes. In a study of morphological and cytological behaviour in ladybird hybrids, crosses have been made between 2-spot and 10-spot ladybirds by Dr Majerus. Cytological behaviour in the sterile hybrids fell into two patterns: (a) meiotic inhibition with chromosome clumping (b) chromosome fragmentation of the type seen in *Drosophila* hybrid dysgenesis (Henderson, unpublished). Abnormalities of testis development comparable to those seen in the *Drosophila* hybrid dysgenesis studies (Thompson, Henderson & Woodruff, 1980), were also found here in the ladybird hybrids.

There is thus good evidence for the existence of hybrid dysgenesis in ladybirds, including the occurrence of chromosome fragmentation, which presumably

involves transposable elements. It is possible that these are involved in the variable sex ratios found, and they may even have been involved in the initial production of the X-autosomal translocations. They may also have been responsible for the production of the supernumerary (B) chromosomes found in some species, including *Exochomus*.

(b) *The persistence of sex ratio differences*

The present of an excess of females in all four members of the Chilocorini which we have studied and in the unrelated *Anatis ocellata*, suggests that the excess is not an accidental occurrence of a transient nature. It implies that the effect may be widespread throughout the Chilocorini and may affect many other species characterized by a neo-XY sex chromosome mechanism. As a number of grasshopper species are known to have evolved this system of sex determination, it would be of interest to know what sex ratios are found in these species.

Presumably selection could act to restore a 1 : 1 sex ratio in a neo-XY system should it be required, but has not done so because there is some selective advantage in having a slight to moderate excess of females in some populations. Most population biologists have argued, since Darwin first put forward the idea, that there is an inherent advantage for bisexual species, particularly those with a conventional sex chromosome system, in maintaining a 1 : 1 sex ratio (Darwin, 1871; Fisher, 1930). This involves the same expenditure of time and energy by the parents on the two types of offspring and would also ensure equal mating opportunities for males and females and maintain a balanced population. Despite these apparent advantages, sex ratios are known to vary widely from a 1 : 1 ratio, particularly in a number of invertebrate groups, including those capable of supplementing sexual reproduction by parthenogenesis (Karlín & Lessard, 1986; Bulmer, 1986; Hamilton, 1967; Robertson, 1966). Such variants usually, though not invariably, involve the production of an excess of females. These are presumably later able to mate with the smaller numbers of males due to the practice of polygamy by the males.

Ladybirds are known to be sexually promiscuous and males can be seen to mate frequently with more than one female. Perhaps the small increased percentage of females present in ladybird species, coupled with the promiscuity of the males, enables larger numbers of eggs and offspring to be produced by a given number of individuals within a population whose size is limited by the availability of highly variable Aphid and Coccid food resources.

(c) *The presence of population differences*

The discovery that all populations of *Exochomus* do not have identical sex ratios is of importance in suggesting that we are not dealing with a static system established by chance and remaining immutable. Rather, it indicates that the sex ratio in this species can, and does, vary in different populations. Such variation, subject to selection, must be meaningful and related to the biological needs or circumstances of the population, although at this stage we do not know what they are. These species of ladybird could provide a valuable source of material for population genetic studies of sex ratio in the future, but the technical problems involved in the field work would be, as we have discovered, formidable.

All attempts which we have made to produce meaningful correlations between the differences in sex ratio found in *Exochomus* and the ecological differences

associated with each population have so far failed to produce satisfactory results. The differences are not directly related to geographical distribution, size of forest, species of tree, nor species on which they are feeding. There are exceptions to all of the possible correlations tested. Thus, there is a tendency for a 50% sex ratio to be found on young pines, such as those found at Lakenheath and Peterborough, and for an excess of females to be found on large, old Douglas Firs, such as those at Swaffham and Brooks Green. But this is confounded by the 50% found at Sandy, on one of the largest, oldest (though solitary and isolated) Douglas Firs in the country.

A great deal more field work, coupled with laboratory breeding and inter-population crosses, will be necessary before the underlying mechanism and selective advantage for this system can be revealed. If a transposable element of the hybrid dysgenesis type is involved, population differences may reflect the distribution of different forms of the element or different strengths of the Y-associated factor.

To conclude this discussion on sex ratio in ladybirds two further topics of interest and relevance should be raised. These involve the presence in one of these species of supernumerary (B) chromosomes and the possible effects of cannibalism on sex ratio.

(i) *Correlated B chromosome polymorphism*

In one of the species examined during the course of these studies, *Exochomus quadripustulatus*, a single large B chromosome was present in some individuals at male meiosis. In only 14 of the 32 populations sampled could estimates be made of the frequency present. This was found to vary from 6.4% to 28.6%. When comparisons were made between the sex ratios present in those populations and their B frequency a linear regression was found, the percentage of B chromosomes increasing as the numbers of males decreased (Henderson, 1988). The presence of variable sex ratios in *Anatis* and the *Chilocorus* species, where B chromosomes were absent, suggests that the B chromosomes are not responsible for affecting the sex ratios in *Exochomus*. Rather the reverse is likely to be true—those factors affecting sex ratio in all species are also likely to be responsible for affecting the frequencies of B chromosomes in different populations.

(ii) *Sex ratio and cannibalism*

Most ladybird larvae are carnivorous, feeding normally on their Aphid or Coccid diets. If food supplies run short, many species, particularly the more voracious such as *Anatis* turn to cannibalism and will eat their smaller brethren. As females tend to be slightly larger than males this could, in theory, lead to an excess of females in starving populations. It could also produce spurious fluctuations in sex ratio from site to site and year to year. We do not believe that cannibalism is responsible for any of the major differences in sex ratio which we have reported in this paper, but it might have affected some of the smaller fluctuations and differences which we have observed. Only a comprehensive further study devoted to this end could determine whether cannibalism is found in these populations and whether, if found, it does modify sex ratios.

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