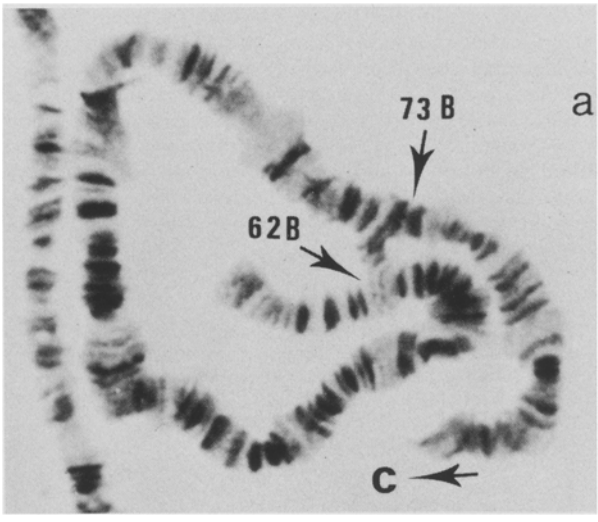

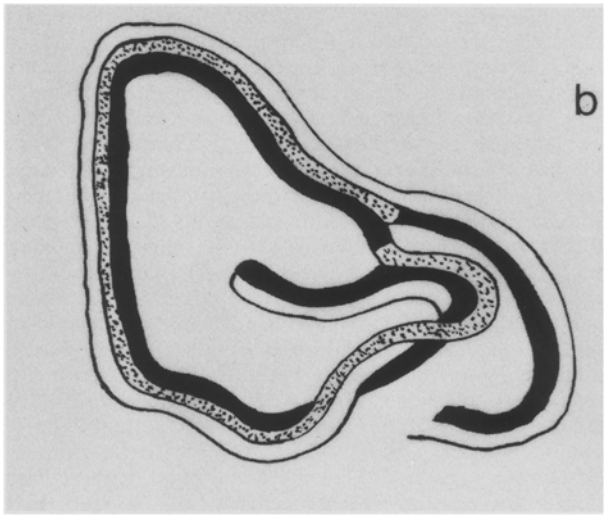

a A photograph of polytene chromosome configuration of a heterozygote for chromosome arm 3L of *D. kikkawai* shows a long segment of tandem duplication. The limits of the 2 break points are clearly visible. Centromeric end (C) is indicated by an arrow. Photograph was taken on Kodak Panatomic-X film with green filter. × 200. *b* A diagrammatic representation of the pairing figure of the triplo. White and black lines represent standard sequence; shaded line is the repeated segment.

There is evidence suggesting that a tandem duplication tends to reduce recombination along the chromosome length[6-8]. On the other hand, there are some suggestions that duplication appears to facilitate chromosome pairing and thus promotes crossing over in the neighbourhood of the repeated segment[9].

A spontaneous tandem duplication involving a considerably long segment of a chromosome is extremely rare. Indeed, such an aberration has been detected only once in several hundred flies from the wild and from the culture stocks in this study. Hence, this observation may serve as an example of this rare phenomenon in eukaryotes.

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2 S. Kitthawee and V. Baimai, J. Sci. Soc. Thailand *5*, 168 (1979).
3 S. Roy and S.C. Lakhotia, Dros. Inf. Serv. *52*, 118 (1977).
4 C.P. Swanson, Cytology and Cytogenetics. Prentice-Hall, Englewood Cliffs, NJ, 1957.
5 A.H. Sturtevant, Genetics *10*, 117 (1925).
6 P.A. Roberts, Genetics *54*, 969 (1966).
7 E.H. Grell, Genetics *50*, 251 (1964).
8 T. Dobzhansky, Z. indukt Abstamm.- und VererbLehre *68*, 134 (1934).
9 M.M. Green, Genetica *33*, 154 (1962).

What determines the population size of the intracellular algal symbionts in the digestive cells of green hydra?[1]

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Summary. The ratios of algal volume to amount of host protein in 5 strains of green hydra were found to be closely similar. However, the components of algal volume varied in the 5 strains, suggesting differences in interactions between animal and algal growth rates.

Algal-invertebrate symbioses exhibit a dynamic equilibrium between numbers of symbionts and amount of available host tissue[2-4]. Perpetuation of a symbiosis depends on maintenance of this balance, for if growth rates of the partners were not identical, one would outgrow the other[5]. The freshwater coelenterate green hydra maintains a population of *Chlorella* algae within its digestive cells. In normal culture conditions, the growth rates of the algae and the animal host are identical, and numbers of algae per digestive cell remain constant[6-8], suggesting that there is some active process whereby stability between the components of the symbiosis is achieved. McAuley[9] has shown that the animal digestive cells control the division of the algae which they contain, but the problem remains of what factors determine the size to which the host permits the symbiont population to grow. In this study, investigations were made to see if variations in algal populations in 5 strains of green hydra could be correlated with a particular quantitative characteristic of the host.

Materials and methods. 3 strains of green hydra (Frome, Jubilee and Coronation) were isolated from the River Frome, Bristol, England, in 1976, 1977 and 1978 respectively. 2 further strains, Florida and European, were kindly supplied by Dr L. Muscatine (University of California, Los Angeles). The European strain was originally isolated in 1966 from a pond near Reading, England; the Florida strain was isolated in Florida, USA. Cultures of hydra were grown in small dishes containing 'M' solution[10], but minus Tris buffer, at 15 °C with 12 h light/12 h dark photoperiod and a light intensity of 1200 lx, in a Gallenkamp illuminated incubator. Hydra were fed on Monday, Wednesday and Friday on freshly hatched nauplii of *Artemia salina*[11].

Standard hydra, each bearing 1 advanced bud, were used throughout.

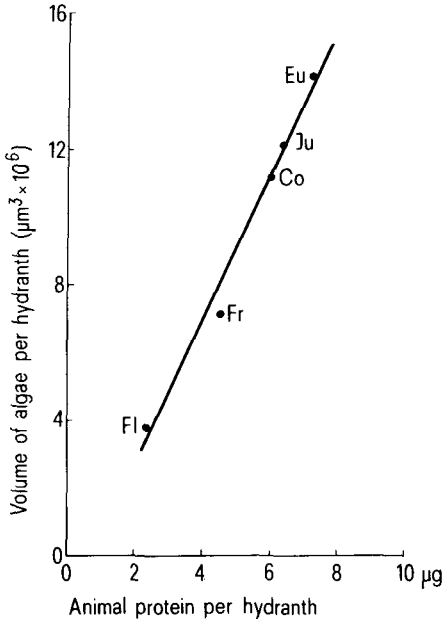
Samples of 10 standard hydra were separated into animal and algal fractions by homogenisation in 1 ml 'M' solution in a glass micro-tissue homogenizer and centrifugation of the resulting suspension at 100 × g for 5 min. This pelleted the unharmed intact algal calls but left the animal homogenate in suspension. The latter was removed and the amount of animal protein determined by the method of Lowry et al.[12]. Microscopic examination of the suspension of animal protein showed that it contained few algae (less than 2% of the total algal population). An additional washing of the algal pellet by resuspension and recentrifugation did not significantly increase the amount of animal protein recovered, but caused a significant loss of algae (~ 10%).

Numbers of algae were determined by resuspending the algal pellet in 1 ml 'M' solution and counting aliquots in a 'Neubar' improved counting chamber. Algal cell diameters were measured directly using a calibrated eyepiece and × 1000 oil immersion optics.

The effectiveness of this separation method was determined by homogenizing and centrifuging different numbers of Jubilee hydra and estimating amounts of animal protein and numbers of algae. Regression analysis showed that there was a significant linear relationship between numbers of algae and amount of animal protein (r = 0.995). This indicates that within the limits of measurement the method gives a good estimate of the algal and animal quantitative relationship in Jubilee hydra, regardless of sample size, and it is assumed that this also applies to the other strains of hydra used here.

Results and discussion. The quantitative characteristics measured were algal numbers per hydranth, algal cell diameter, and amount of animal protein per hydranth. The table summarizes the measurements of these characteristics in the 5 strains of green hydra. There is no clear relationship between host size (as measured by amount of protein) and either numbers of algae or size of algal cells. Thus, Frome hydra, although smaller than Jubilee and Coronation hydra, contain similar numbers of algae, and Jubilee algae are the largest although their hosts do not contain the most animal protein.

However, a highly significant correlation was found between amount of host protein and volume of symbiont population (figure). Mean algal cell volume was calculated using the formula $^4/_3\ \pi r^3$ (assuming the algae to be perfect spheres), and this was multiplied by the number of algae per hydranth to give the total volume of algae per hydranth. The correlation obtained shows that, in the given culture conditions, the 5 strains of green hydra support similar volumes of algae per unit tissue. This suggests that the host restricts the amount of space available to the symbionts, and may be related to the observation of McAuley[9] that the animal digestive cells control the division of the algae they contain. However, the balance between host and algal population size (as measured by volume) appears to be achieved in different ways in the 5 strains; hydra may contain many small or fewer large algae per unit tissue. Thus, although the 5 strains studied here exhibit closely similar ratios of algal volume to amount of host protein, variation in algal numbers and cell size suggest that these similar ratios are the products of interactions between animal and algal growth patterns. Experiments involving cross infection of aposymbiotic hydra with algae of different strains[13] and examination of the resulting balance between the host and newly introduced symbionts may show whether host or algal characters are the chief determinants of these variations. It would also be valuable to investigate how the balance between host and symbiont changes with alteration in environmental conditions such as temperature, photoperiod and light intensity.



Linear correlation of animal size and total algal volume in 5 strains of green hydra. Fl, Florida; Fr, Frome; Co, Coronation; Ju, Jubilee; Eu, European. r = 0.995, y = 2.17x − 1.54.

Total numbers of algae, algal diameter and amount of animal protein in standard animals of 5 strains of green hydra grown at 15 °C

Strain	Algae per hydranth × 10^5	Algal diameter (μm)	Animal protein per hydranth (μg)
Frome	0.93 ± 0.08	5.27 ± 0.84	4.37 ± 0.22
Jubilee	1.12 ± 0.06	5.93 ± 0.91	6.26 ± 0.99
Coronation	1.27 ± 0.13	5.62 ± 0.91	6.01 ± 1.01
Florida	0.66 ± 0.07	4.80 ± 0.56	2.30 ± 0.26
European	1.57 ± 0.10	5.56 ± 0.65	7.20 ± 0.51

Figures are amalgamated means ± 1SD. Algae per hydranth and animal protein per hydranth determined from 5 replicate samples each of 10 standard hydra; algal diameters from measurements of 100 cells in each of 3 replicate samples of 10 standard hydra.

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2 L. Muscatine, in: The biology of Hydra and other coelenterates, p. 255. Ed. H.M. Lenhoff and W.F. Loomis. University of Miami Press, Miami 1961.
3 D.L. Taylor, J. mar. biol. Ass. U.K. *49*, 1057 (1969).
4 L. Muscatine and R.R. Pool, Proc. R. Soc. Lond. B. *204*, 131 (1979).
5 M. Droop, Symp. Soc. gen. Microbiol. *13*, 171 (1963).
6 R.L. Pardy, Biol. Bull. *147*, 105 (1974).
7 R.R. Pool, Ph.D. Thesis, University of California, 1976.
8 R.L. Pardy and L. Muscatine, Biol. Bull. *145*, 565 (1973).
9 P.J. McAuley, J. Cell Biol. *47*, 197 (1981).
10 L. Muscatine and H.M. Lenhoff, Biol. Bull. *128*, 415 (1965).
11 W.F. Loomis and H.M. Lenhoff, J. exp. Zool. *132*, 555 (1956).
12 G.H. Lowry, N.H. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
13 E. Jolley and D.C. Smith, Proc. R. Soc. Lond. B. *207*, 311 (1980).