THE DISPERSiON OF ECHINOCOCCUS GRANULOSUS IN THE INTESTINE OF DOGS

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ABSTRACT: We studied the dispersion of adult Echinococcus granulosus in the intestine of experimentally infected dogs at 2 scales of habitat use. On a coarse scale, worms were found most frequently in the anterior third of the small intestine. On a fine scale, clumps or aggregations, typically of 4-5 worms in an area of 12 mm², occurred throughout the anterior two-thirds of the intestine. The most likely proximate cause of aggregative behavior is attraction between individual worms. There are at least 2 equally plausible ultimate causes of the behavior: to enhance cross-fertilization and to improve the quality of the environment. Restriction of worms to the anterior small intestine may be a consequence of aggregative behavior on a finer scale or a response to different proximate and ultimate factors.

All species of parasites are restricted, not only to particular host species, but also to specific sites on or in the host. Site specificity is usually presumed to result from active site selection by the parasite (Holmes, 1973). Identification of the causes of site selection requires a distinction between proximate and ultimate explanations. The proximate causes are those factors that act as the immediate stimulus for the behavior. These usually are presumed to be specific physical or chemical cues, although there is little supporting evidence (Mettrick and Podesta, 1974; Sukhdeo and Mettrick, 1987). The ultimate causes of site selection are those factors responsible for the evolution of the behavior. Site specificity implies restriction along 1 or more dimensions of the niche of a species and there has been much speculation on the function of niche restriction in parasites. Price (1984) argued that species of parasites are highly specialized to exploit precise microenvironments and that niche restriction is therefore a consequence of choosing an optimal habitat. Holmes (1973) presented evidence, largely from cestodes and nematodes, that interspecific competition is responsible for niche restriction. Rohde (1979) and Rohde and Hobbs (1986) argued, primarily from data on monogeneans, that niche restriction functions to enhance intraspecific contact and mating opportunities in low-density populations.

Different processes may be responsible for site selection in different species. Even in 1 species, there may be several proximate and ultimate

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of individual worms attached to the intestinal wall of experimentally infected dogs. This may provide a more appropriate scale at which to examine the proximate and ultimate causes of site selection in the species.

MATERIALS AND METHODS

Collection of protoscoleces

Protoscoleces were removed from hydatid cysts obtained from naturally infected sheep, cattle, and kangaroos (Macropus fuliginosus). Protoscoleces from 1 or more cysts in the same organ of an individual host were regarded as 1 isolate. Nineteen isolates were obtained from 4 states and territories throughout Australia; 5 from Victoria, 5 from the Australian Capital Territory, 4 from New South Wales, and 5 from Western Australia. All isolates conformed morphologically to the Australian mainland sheep strain of E. granulosus, as defined by Kumaratilake and Thompson (1984).

Infection of dogs

Each isolate was used to infect 1 or more dogs; 26 dogs were infected from 19 isolates. Domestic cross-breeds of both sexes were used. At the time of infection, they ranged in age from 3 to 12 mo. The history of previous infections was not known. On admission to the School of Veterinary Studies, Murdoch University, all dogs were treated with praziquantel to remove adult tapeworms and their feces were examined regularly thereafter for the presence of tapeworm eggs. Each dog was infected orally with either 0.1, 0.2, 0.3, 0.4, 0.5, or 0.8 ml of protoscoleces, washed in phosphate-buffered saline, and packed in a gelatin capsule (0.1 ml is equivalent to approximately 40,000 protoscoleces [Thompson and Kumaratilake, 1985]). We obtained isolates at irregular intervals and infections took place over a 2-yr period. Despite this, all infections were carried out under standard conditions, immediately before feeding. After infection, all dogs were housed in similar conditions and maintained on a standard diet of tinned dog meat, dry biscuits, and water ad lib.

Autopsy and measurement of dispersion

Thirty-five days after infection, dogs were killed by an injection of pentobarbitone sodium, and the entire small intestine was removed. The intestine was opened longitudinally along the line of mesenteric attachment and divided transversely into thirds. A 50-mm length of tissue was removed from the anterior edge of each third, pinned to a piece of foam, and fixed in 10% formalin. The remainder of each third was incubated separately in a beaker of Hanks' balanced salt solution for 30 min at 37 C. The number of worms in each third of intestine was counted directly or estimated volumetrically.

Fixed intestinal sections, pinned to foam, were photographed and enlarged to approximately 5 times their natural size. Photographs were overlain with a transparent grid of 5-mm x 5-mm contiguous quadrats and the number of worms in each quadrat scored. Worms were scored as belonging to a quadrat if more than half of their strobila was contained within it. Quadrats in which worms were obscured, such as those along the edges of intestinal sections, were ignored in all analyses.

Analysis

Data from quadrat samples were used to measure the spatial pattern of worms attached to the intestinal wall. Several indices have been devised to describe, from quadrat counts or distance measures, the pattern or arrangement of individuals in a population and to test the departure of this pattern from randomness (see Patil and Stieeler, 1974; Southwood, 1978). All indices have limitations; we used the following 4 techniques because they differed in their assumptions and enabled the data to be analyzed in slightly different ways.

1) Negative binomial distributions were fitted to the data by the maximum likelihood method (Bliss and Fisher, 1953) and goodness of fit tested by x2. Aggregated patterns often follow the negative binomial distribution and the exponent k is used as an index of aggregation; the smaller the value of k, the greater the extent of aggregation (Southwood, 1978). The mean size (number of individuals) of a clump in a negative binomial distribution was calculated as \( \lambda = (x/2k)V \) (Arbous and Kerrich, 1951), where \( x \) is the mean number of worms per quadrat and V is a \( x^2 \) value at the 0.5 probability level with 2k degrees of freedom.

2) Morisita’s test statistic (Morisita, 1959) was calculated as \( \hat{I}_0 = q \sum_{i=1}^{n} n_i(n_i - 1)/N(N - 1) \), where q is the number of quadrats, \( n_i \) is the number of individuals in the ith quadrat, and N is the total number of individuals. For a random pattern \( \hat{I}_0 = 1 \), values of \( \hat{I}_0 > 1 \) imply an aggregated pattern and values of \( \hat{I}_0 < 1 \) imply a regular pattern. The probability of observed departures from randomness can be obtained from \( F_0 = [I(N - 1) + q - N]/q - 1 \), with q - 1 and \( \infty \) degrees of freedom.

3) Moran’s coefficient of autocorrelation (Moran, 1950) was calculated as \( I = [q \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} z_i z_j]/(S_0 \sum_{i=1}^{n} z_i^2) \), where q is the total number of quadrats, \( w_{ij} \) is the weight of connection between the ith and jth quadrats, \( z_i = n_i - \bar{n} \) (\( n_i \) is the number of individuals in the ith quadrat), and \( S_0 = \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} \). Values of \( w_{ij} \) were set to 1 for pairs of quadrats with adjacent edges, 0.5 for pairs with adjacent vertices, and 0 for all other pairs. I varies from \(-1 \) to \(+1 \), with the expected value in the absence of autocorrelation approaching 0 for large sample sizes; values of I greater than expected imply positive autocorrelation (adjacent quadrats with similar numbers of individuals), values less than expected imply negative autocorrelation (adjacent quadrats with dissimilar numbers of individuals). Standard deviations were calculated to measure the significance of departure from expectation (Cliff and Ord, 1981).

4) Taylor’s power law (Taylor, 1961), \( s^2 = ax^b \) or \( \log s^2 = \log a + b \log x \), where x is the mean number of individuals per quadrat (density) and \( s^2 \) is the variance, was used to describe the relationship between dispersion and density over all samples. The slope of the regression (b) indicates the rate of increase of vari-
ance as mean density increases and is considered to be a measure of the density dependence of aggregation (Taylor et al., 1978, but see Downing, 1986). If \( b = 1 \), aggregation does not change with density, if \( b > 1 \) the population becomes more aggregated at higher densities and if \( b < 1 \), the population becomes more regular at higher densities. Because both variance and mean density are subject to error, their relationship should be calculated as a functional, geometric mean regression. In practice, however, this more accurate approach is seldom required (Southwood, 1978), and we have used predictive least squares regression analysis.

These 4 measures were obtained for a range of quadrat sizes by combining adjacent quadrats and calculating number of individuals per quadrat. This produced a series of 8 data sets per sample for fitting the negative binomial, Morisita's index and Taylor's power curve, and 5 data sets/sample for spatial autocorrelation. Because quadrats along the edges of intestinal sections were not used, analyses did not account for 3-dimensional structure. This will introduce errors into all measures when using data from combined quadrats.

**RESULTS**

**Recovery of adult worms**

Adult worms were recovered from 23 infected dogs (88%). There was a significant linear relationship between the logarithm of the number of adult worms recovered and the volume of protoscoleces ingested (\( \log Y = 2.46 + 1.91X, P (\beta = 0) < 0.05 \)), although it accounted for only a small proportion of the variance in recovery (\( r^2 = 0.18 \)).

**Dispersion of worms between intestinal segments**

The number of adult worms in each third of the small intestine was scored in 19 infected dogs. In all cases, there were significant differences in the number of worms between segments \( (x^2, P < 0.001) \). The mean proportion of worms in each segment was 0.69 in the first (most anterior), 0.27 in the second, and 0.03 in the third. Dispersion differed between dogs (heterogeneity \( x^2 = 31,007, P < 0.001 \)), with 15 dogs having the majority of worms in the first segment and 4 dogs having most worms in the second segment. Differences in dispersion did not appear to be related to sex or age of the hosts. There was no significant relationship between the total number of worms recovered and the proportion in each intestinal segment (Spearman’s rank correlation: for the first segment \( r_s = 0.13, P > 0.50 \); for the second \( r_s = 0.18, P > 0.20 \); for the third \( r_s = 0.25, P > 0.20 \)).

**Dispersion of worms within intestinal segments**

The spatial pattern of worms was measured in 24 sections of intestine from 16 dogs. Fourteen sections came from the first segment of intestine and 10 from the second. All indices of dispersion indicated an aggregated pattern at the smallest quadrat size. Morisita’s I\( \delta \) was significantly greater than 1 in all samples. Moran’s I was significantly greater than expected in 20 samples and showed the same trend in the other 4 samples. The negative binomial provided an adequate fit to the data from 22 samples. There was no evidence of difference in the degree of aggregation between samples from the first and second segments of intestine, or between dogs of different sex or age.

In general, I\( \delta \) decreased and k of the negative binomial increased as quadrat size increased. Morisita (1959) calculated the relationship between I\( \delta \) and quadrat size for various theoretical spatial patterns (see inset, Fig. 1). Plots from our data were of 2 types. Eighteen samples were consistent with Morisita’s pattern for an aggregated dispersion with random intraculum spacing, whereas 6 samples conformed to the pattern for an aggregated dispersion with regular intraculum spacing. Plots for representative samples of each type are shown in Figure 1.

We used changes in spatial autocorrelation with quadrat size to estimate the size and area covered by a clump. Plots of I against quadrat size were of 3 types. In the most common pattern, shared by 12 samples, I remained constant or increased slightly over the first 3 quadrat sizes, then fell rapidly toward expected values. In 7 samples, I increased gradually and in the remaining 5 samples decreased gradually, over all quadrat sizes. Plots for representative samples are shown in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Plot of Morisita’s index (I\( \delta \)) against quadrat size (expressed as intestinal area, in mm\(^2\), after correction for photographic enlargement) for 2 samples of *Echinococcus granulosus* from the intestine of dogs. Open symbols indicate values significantly greater than 1. Inset: relationships between I\( \delta \) and quadrat size for theoretical spatial patterns; random, regular, aggregated with intraculum dispersion random, aggregated with intraculum dispersion regular.
Figure 2. We took the quadrat size at which the last significant value of I occurred as a measure of the area covered by a clump. After correction for photographic enlargement, this ranged from 1 mm$^2$ to 25 mm$^2$, with a mean of 12.4 mm$^2$ (SE = 1.8 mm$^2$). The negative binomial distribution provided an adequate fit for the data from 22 samples at these quadrat sizes and the mean number of worms per clump, calculated by the method of Arbous and Kerrich (1951), ranged from 0.1 to 21.9, with an overall mean of 4.5 (SE = 1.2).

The sections of intestinal tissue that were used to measure spatial pattern were approximately 2,500 mm$^2$ in area. With an average area/clump of 12.4 mm$^2$, there were often several clumps in each section. These clumps could themselves exhibit a spatial pattern. It is difficult to obtain quantitative measures of such second-order patterns, but examination of original photographs and density maps computed from quadrat counts suggested a random dispersion of clumps in sections of intestine (Fig. 3).

To determine the relationship between aggregation and density, we plotted values for our aggregation indices against mean number of individuals per quadrat for each sample. Although $I_0$ was negatively related to mean density, Iwao (1968) considered this an inherent property of the index. Moran’s I was positively related to mean density; Figure 4 shows this relationship for the smallest quadrat size. This increase in degree of aggregation with increasing density was confirmed by Taylor’s power law. Log variance was significantly related to log mean density at all quadrat sizes, with a positive intercept indicating aggregation. The slope, b, was significantly greater than 1 at all quadrat sizes except the smallest (Fig. 5).

**DISCUSSION**

Adult *E. granulosus* exhibited spatial pattern on 2 scales of habitat use. On a coarse scale, worms were found most frequently in the anterior third of the small intestine of infected dogs; we will refer to this as site restriction. On a finer scale, clumps of worms were found attached to the wall throughout the anterior two-thirds of the intestine, a phenomenon we refer to as aggregation. Site restriction and aggregation occurred in all dogs, regardless of sex or age. Site restriction may have been a consequence of the probability of attachment of evaginated protoscoleces as they travelled along the intestine. This seems unlikely, however, because both the present study and that of Macpherson et al. (1985) found major differences in dispersion of worms between intestinal segments in different dogs under identical conditions of infection. Similarly, it is possible that aggregation resulted from clumping of protoscoleces within hydatid cysts, but the protoscoleces should have been removed from their brood capsules and thoroughly dispersed, both by washing prior to infection and by the action of pepsin in the stomach (Smyth, 1969). Presumably then, site restriction and aggregation resulted from the behavior of developing worms. We will attempt to infer the proximate and ultimate causes of this behavior, proceeding from finer to coarser scales of habitat use.

The proximate cause of aggregative behavior may be either attraction between individuals or attraction to patchy microenvironmental factors. Clumps of about 4–5 individuals occurred throughout a substantial portion of the small intestine. They occupied an area of about 12 mm$^2$ and there was no observable second-order pattern to their occurrence within sections of intestine. These data are more consistent with attraction between individuals than with attraction to environmental factors, especially if structural and physicochemical gradients in the intestine of vertebrates are essentially linear, as proposed by Read (1971), Crompton (1973), and Mettrick and Podesta (1974).

The ultimate cause of aggregative behavior in *E. granulosus* is more difficult to infer. At least 5 biological functions of such behavior have been suggested for other organisms; choice of an optimal habitat (Price, 1984), avoidance of pre-
dation (Bertram, 1978), avoidance of interspecific competition (Holmes, 1973), enhancement of mating opportunities (Rohde, 1982), and improvement of environmental quality (Way and Cammell, 1970). Three of these can be ruled out for E. granulosus. Firstly, because of the small size of clumps and their occurrence throughout the small intestine, aggregative behavior is unlikely to result in optimal habitat use. Secondly, we are not aware of any predators (or parasites) that could have been responsible for the evolution of the behavior. Thirdly, the occurrence of aggregations in the absence of other species indicates that aggregative behavior is not primarily an interactive response to interspecific competition. It may be regarded as a selective response if the behavior minimizes niche overlap between E. granulosus and other species of intestinal helminths. This is unlikely, however, if the proximate cause of the behavior is attraction between individuals, because the broad fundamental niche of E. granulosus means that the presence of conspecifics will not provide a reliable cue to the absence of interspecific competitors. Kuno (1988) showed that increasing the patchiness of a species distribution relaxes interspecific competition, even when 2 species share the same niche. In his model, however, this occurs because of a lowering of equilibrium density through increased intraspecific competition within patches. Aggregative behavior could not have evolved through individual selection to enhance intraspecific competition, although that may well be an effect (sensu Williams, 1966) of the trait.

The observed spatial pattern and likely proximate cause of aggregative behavior in E. granulosus are consistent with both of the remaining functions. Enhancement of mating opportunities requires that cross-fertilization occurs between worms. This has never been observed directly in E. granulosus, although self-fertilization (or at least self-insemination) has been reported in a number of studies (Kumaratilake et al., 1986). Data on the genetic variation of E. granulosus in Australia suggest that both cross- and self-fertilization may occur in natural populations (Lymbbery and Thompson, 1988). Environmental improvement could take the form of changes in gut morphology near groups of worms, providing easier attachment, or of changes in nutrient concentrations through increased flow in the intestinal lumen or increased breakdown of mucus. However, we are not aware of any evidence that such changes occur in hosts infected with E. granulosus.

The degree of aggregation increased with increasing density of worms in the intestine. Taylor and Taylor (1977) interpreted this phenomenon, which has been observed in a wide range of free-living species, as evidence that the function of aggregative behavior is to maximize environmental quality. Anderson et al. (1982) and

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**Figure 3.** a. Photograph of a section from the first third of the small intestine from a dog, 35 days after infection with 0.5 ml of protoscoleces of Echinococcus granulosus. Scale bar = 10 mm. b. Density map, computed from the photograph by counting the number of worms in 1-mm² quadrats.

**Figure 4.** Relationship between Moran’s I and mean density (number of worms per quadrant) at the smallest quadrat size for 24 samples of Echinococcus granulosus from the intestine of dogs. Regression line described by log Y = −0.73 + 0.40 log X (r² = 0.41, P (β = 0) < 0.001).

**Figure 5.** Slope (b) of the regression of log variance on log mean density at different quadrat sizes (expressed as intestinal area, in mm², after correction for photographic enlargement). b is significantly greater than 0 at all quadrat sizes and significantly greater than 1 at all except the first.
Downing (1986), however, pointed out that density dependence of aggregation may arise from the stochastic interplay of demographic events and environmental heterogeneity, and it need not imply an evolved behavioral response.

Although the observed relationship between the degree of aggregation and density cannot be used to support either of the likely ultimate explanations of aggregative behavior in *E. granulosus*, these explanations do make different predictions about the degree of aggregation in response to development of worms and genetic heterogeneity of the initial infection. If aggregative behavior increased around the time of sexual maturity, this would be consistent with the enhancement of mating opportunities but not with improvement of environmental quality. Although there is no evidence for extensive migrations in the intestine, worms are able to move between adjacent villi as soon as their hooks and suckers can be used for attachment (Smyth et al., 1969). Sexual maturity is reached about 28 days after infection with the sheep strain of *E. granulosus* (Smyth et al., 1969) and there is evidence that movement declines soon afterwards (Thompson et al., 1979). We have attempted recently to measure spatial pattern in dogs autop-sied 20 and 25 days after infection, but worms were too small to be visible consistently above the villi of the intestine. If the function of aggregative behavior is to enhance mating between worms, and if cross-fertilization serves to promote outbreeding, then the degree of aggregation may be expected to be greater in genetically heterogeneous populations than in populations of genetically identical individuals derived from a single cyst (assuming that worms are capable of distinguishing different genotypes). This would not be predicted if the function of aggregative behavior is to improve environmental quality. We found no difference in the degree of aggregation between infections derived from a single cyst and those derived from a number of cysts from the same host, but we have no measure of genotypic diversity in the latter case.

As well as forming clumps on the wall of the small intestine of infected dogs, adult worms occurred disproportionately along the length of the intestine. This site restriction may be a by-product of aggregative behavior on a finer scale. Alternatively, the proximate factors responsible for site restriction may operate independently of those causing aggregative behavior. The best evidence that different factors are responsible for spatial pattern at different scales of habitat use comes from studies that have reported a decrease in site restriction at higher densities (Sweatman and Williams, 1963; Macpherson et al., 1985; Rausch, 1985; Gemmell et al., 1986). If this occurs, it must be through processes operating independently of those on a finer scale because we found that the degree of aggregation increased with density. An analysis of previous studies, however, shows that there is no published evidence to support a relationship between density and site restriction for *Echinococcus*. Most reports are anecdotal. Macpherson et al. (1985: table 3) provided data (pooled from a number of experimentally infected dogs) on the percentages of worms in 4 regions of the small intestine. Although pooling may have obscured trends in individual dogs, there is no significant relationship between total number of worms recovered and percentage in each region of the intestine (Spearman's rank correlation: for the first region \( r_s = 0.50, P > 0.05 \); for the second \( r_s = 0.30, P > 0.10 \); for the third \( r_s = 0.43, P > 0.05 \); for the fourth \( r_s = 0.37, P > 0.10 \). In the present study, we also found no relationship between number of worms in the intestine and dispersion between intestinal segments.

Rausch (1985) suggested that the restriction of adult *Echinococcus* to certain regions of the small intestine of dogs functions to enhance cross-fertilization. However, until it can be shown that site restriction is not simply a consequence of aggregative behavior, a search for ultimate explanations of the phenomenon seems premature. We believe that it would be more rewarding for further analyses of the dispersion of *E. granulosus* in the intestine of dogs to concentrate on the finer scale of habitat use.

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**LITERATURE CITED**


