MANIPULATIONS OF EGG-GALLERY LENGTH TO VARY BROOD DENSITY IN SPRUCE BEETLE *DENDROCTONUS RUFIPENNIS* (COLEOPTERA: SCOLYTIDAE): EFFECTS ON BROOD SURVIVAL AND QUALITY

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Abstract

Different brood densities were produced under a constant bark surface area of the spruce host, by excising egg-producing female *Dendroctonus rufipennis* from the host material after they had excavated galleries of specified lengths. This procedure allowed a constant attack density. The numbers of adult progeny produced/cm of egg-gallery were significantly greater from bark slabs with short galleries and low densities: the sizes (pronotal widths) of adult progeny of both sexes were also significantly greater from low than from high densities; and the distribution patterns of chromatin differed significantly among high, medium and low densities.

Résumé

Les auteurs ont obtenu différentes densités d'oeufs sous une section constante d'écorce de l'épinette hôte en retirant des femelles ovipares de *Dendroctonus rufipennis* du tissu hôte après qu'elles aient creusé des galeries de longueur déterminée. Cette méthode permet d'obtenir une densité d'invasion constante. Le nombre de descendants adultes produit par centimètre de galerie de ponte était beaucoup plus élevae dans les sections d'écorce à galeries courtes et à densité d'oeufs faible; la taille (largeur du pronotum) des descendants adultes des deux sexes étaient également beaucoup plus élevée lorsque la densité des oeufs était faible. De plus, les modes de répartition de la chromatine différaient énormément selon qu'elle provenait d'oeufs à forte, moyenne ou faible densité.

Introduction

In population dynamics studies of bark beetles, the effect of density on brood production has been studied mostly in relation to the attack density of the parent beetles (McMullen and Atkins 1961; Cole 1962; Reid 1963; Berryman and pienaar 1973). Studying this relationship carries practical advantages in that the attack density can be readily determined in natural stands without destroying the sample and variations in attack density can be easily created in controlled experiments. Such experiments, however, do not discriminate between reduced brood production due to fewer parent beetles (McMullen and Atkins 1961; Thomson and Sahota 1981) and that due to brood density and competition among the progeny. Some experiments, using this approach, have related brood quality, such as adult size in the brood, to the density of parental attack (Reid 1962; McGhehey 1971; Safranyik and Linton 1985). Possibly, the effect of attack density on brood is indirect, emanating through changes in brood density.

Cole (1973) isolated the effects of brood density on the quality of the brood as shown by their reproductive capacity when mature. Varying the density of *Dendroctonus ponderosae* larvae on a phloem diet showed that increased density and competition among larvae led to reduced reproductive capacity in the resulting adult females. In the present paper, we have isolated the effects of brood density from those of parental attack density among progeny raised on the natural host diet. Survival and individual size of the brood adults are reported. Also reported are cytological differences among the brood from the various density classes as indicators of differences in population quality.

Materials and Methods

The spruce beetles, *Dendroctonus rufipennis* (Kirby) used in this study were collected from a natural endemic field population near Hixon, British Columbia as well as the laboratory-reared progeny of these beetles. The bolts of host trees containing adult beetles were collected from the field and stored in the laboratory at O°C (±1°C) from October to April.

In May the bolts were transferred to cages at room temperature leading to emergence of the beetles. Fresh host material was obtained from the beetle collection site by cutting a spruce tree (*Picea engelmannii* Parry) of 34-cm-dbh. Bark-bearing slabs measuring 30 cm x 20 cm x 5 cm thick were cut out of the bole. Surfaces without bark were coated with molten paraffin wax to avoid excessive moisture loss.

Each female beetle was introduced 5 cm from one end of each slab by making a small hole in the bark and confined there with a gelatin capsule. The male followed the female one day later. Infested slabs were maintained at 18.3 ± 1°C, each standing on its beetle-containing end to encourage directional gallery production by the beetles. This arrangement was used to produce three sets of slabs (9-10 slabs/set) containing parental galleries of 6.8 cm, 9.8 cm and 12.8 cm while the bark surface areas of all the slabs remained identical. The required gallery length was achieved by X-raying the slabs and excising the female at the proper point. Beetle excision was carried out by cutting about 1 cm x 1 cm piece of the bark. This opening was sealed with molten paraffin after removal of the female. It was not possible, however, to excise each beetle at precisely the specified gallery length. Eighteen slabs were started for each density level; 9-10 of these were successfully colonized for each level. The three brood densities resulting from these galleries were designated as low, medium and high respectively. The slabs containing the parental galleries and eggs were kept at 18.3°C. In September, when the progeny had reached the adult stage, the slabs were placed at 0°C for over-wintering. The slabs were peeled to remove the adult progeny in May of the following year. The total parental gallery length, the egg-gallery length (total parental gallery - initial egg free gallery) and the number of adults produced in each slab were recorded. The pronotal widths of all progeny were measured and their sexes determined.

Cytological investigations relating to quality differences among progeny from different densities were carried out by analysing digitized images of fat body nuclei (Sahota *et al.* 1984). Ten to 15 females from each density group were fixed in 3 parts ethanol: 1 part acetic acid for 2h. Their abdomens were slit open to facilitate penetration of fixative. The fat body was stained in situ with Fuelgen stain after a 20 min hydrolysis with 3.5N HCl at 37°C. The fat body cells were spread on microscopic slides as described by Farris *et al.* (1982).

Digitized images of fat body nuclei were created by scanning the samples at 570 nm using a Zeiss SMP5 microphotometer system on line to a PDP 11/34 minicomputer. This process measures the light transmitted through every 0.25 µm square of the scanned area producing a matrix of numbers or the digitized image. Materials other than nuclei in the digitized images were removed by editing. Seventy-five variables were mathematically derived from each of the edited images. Derivation of these variables or features along with the scanning and editing procedures are described in Peet and Sahota (1984, 1985) and Sahota *et al.* (1986).

For analysis of data dealing with gallery lengths, progeny produced, and average individual size of the progeny at various densities, we used ANOVA followed by Newman-Keul's multiple range test. The relationship between the adult progeny per unit of egg-gallery length at various densities was examined by regression analysis.

To investigate cytological differences among progeny groups from different densities, the three features with the highest merit value for discriminating among the three density classes were selected by the computer. These included a histogram feature (HIST04) and two transition probability features (TRPR23 and TRPR61). Histogram features examine the probability of the pixels of a nucleus belonging to a given optical density bin of a 20-bin histogram generated from the optical densities of all the pixels comprising the nucleus. HIST04 refers to the fourth bin of such a histogram. Transition probabilities relate to the degree of change of optical density between a pixel and its eight immediate neighbours. A detailed description of these and other features is given in Peet and Sahota (1984). We applied discriminant analysis (Cooley and Lohnes 1971; Duda and Hart 1973) to the above three features of the three cell populations. These methods generated a set of axes that maximized the distances between the distributions representing various populations and minimized the distances within each distribution. The 99% confidence ellipses were drawn with respect to the first two of these new variables as the two axes. A more detailed description of this application

of discriminant analysis is given in Sahota *et al.* (1986).

Results and Discussion

Changes in brood density per 20 cm x 30 cm bark surface created by the variations in the length of egg-gallery resulted in changes in the pronotal widths of brood members as well as their survival to the adult stage. There was a significant decrease ($p < 0.01$) in the pronotal widths with increasing brood density in both sexes (Table 1). This is similar to the results obtained by varying the attack density (Safranyik and Linton 1985). The three groups of egg-gallery lengths created for producing the three brood density classes were significantly different from each other ($P < 0.05$). However, the number of adult progeny per slab or per cm of the egg-gallery differed significantly between high and low densities but neither differed from the medium density ($P > 0.05$) (Table 1).

When adult progeny per unit length of the egg-gallery was plotted against the length of such galleries (Fig. 1), it was shown that increased egg-gallery length and competition led to a decrease of adult progeny produced per cm of this gallery. The relationship depicted in this figure was significant at the 95% confidence level. Thus it appears that the range of brood densities created in the experiment produced biological effects of competition on brood survival. Safranyik and Linton (1985) concluded that fewer adults produced per unit length of the egg-gallery with increasing density was due to brood mortality. In their experiments, however, differences in brood density were created by varying the attack density of parent beetles per unit area of bark surface. Their argument that the decrease in adult progeny per unit gallery length was not due to a decrease in egg production, was based on the demonstration by Thomson and Sahota (1981) that competition among parent beetles does not alter the number of eggs deposited per unit egg-gallery length. The present results are in agreement with those of Sefranyik and Linton (1985) but provide more direct evidence to show that the decrease in adult progeny produced per unit length of the egg-gallery was solely due to brood competition as parental attack density was constant in these experiments. It may be pointed out that in most of the slabs less than 70% of the phloem was used by the brood. In two of the slabs nearly 90% of the phloem had been used.

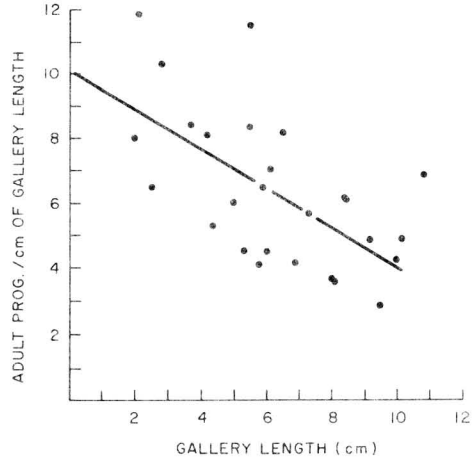

Fig. 1. Influence of egg-gallery length (brood density) on the survival per cm of egg gallery of *Dendroctonus rufipennis* brood in 30 cm x 20 cm of bark surface area.

Table 1. Egg-gallery lengths of the parent adults and some characteristics of their adult progeny raised at three brood density levels on 30cm x 20cm slabs of *Picea engelmanni*.

		Brood Density Level		
		High	Medium	Low
No. of parental egg-galleries		9	9	10
Egg-gallery Lengths (cm)		8.81 a (0.40)	6.01 b (0.02)	3.72 c (0.41)
Adult progeny per bark slab		42.8 ab (4.9)	38.7 bc (5.0)	23.8 c (2.4)
Brood adults produced per cm. of egg-gallery		4.81 ab (0.42)	6.15 bc (0.88)	7.09 c (0.91)
Pronotal widths of brood adults (mm)	Female	2.14 a (0.004)	2.29 b (0.005)	2.38 c (0.01)
	Male	2.09 a (0.003)	2.29 b (0.007)	2.34 c (0.01)

Note: Numbers within brackets show the standard error of the means they are associated with. Means followed by the same letters within each row are not significently different at 95% level (Newman-Keul's multiple range test).

Effects of the brood density on the "population quality" of the progeny were investigated by examining individual size (pronotal width) in the progeny and changes created in the distribution patterns of chromatin (DNA). Increase in brood density created by the increase in length of the egg-galleries resulted in a significant reduction of the individual size of the progeny of both sexes (Table 1). Brood density also produced cytological changes in the fat body nuclei which reveal significant differences among the progeny from the three brood density classes. Fig. 2 shows the 99% confidence ellipses of the means of the progeny from the three density classes. These ellipses are based on the three features with the highest merit value derived from the distribution pattern of chromatin. Chromatin distribution pattern have been used to demonstrate small differences among cell and insect populations (Bartels and Wied 1977; Bartels and Olson 1980; Sahota *et al.* 1984). Furthermore, Sahota *et al.* (1986) have shown that changes in the functions of the differentiating follicular epithelial cells are accompanied by changes in the chromatin distribution patterns and that the treatments leading to blockage of functional differentiation of these cells also block changes in chromatin distribution patterns, thus providing evidence for a relationship between chromatin distribution pattern and cell function.

The results presented in this paper show that the influences of brood density on the survival and quality of the progeny are similar to those created in response to attack density (See Safranyik and Linton 1985). It appears that attack density effects are produced indirectly through brood density as a result of competition. The results also show that analysis of chromatin distribution patterns can detect population quality differences created by brood density and competition. Sahota *et al.* (1984) have pointed out that population quality differences related to reproductive capacity may result from the influence of a variety of factors such as environment, genetics, competition, disease, etc. However, chromatin distribution patterns resulting from the influence of these factors may be different. Thus, density-related distribution patterns of chromatin in broods provide further information required to build a comprehensive picture of chromatin distribution patterns in relation to population quality and reproduction.

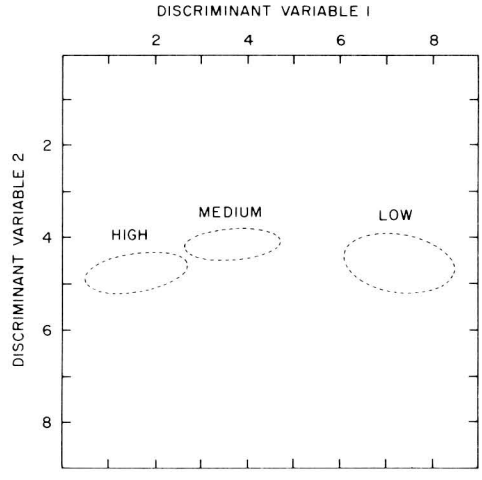

Fig. 2. Ninety-nine percent confidence ellipses of the means of distribution of the *Dendroctonus rufipennis* broods raised under three different densities. These distributions are based on patterns of chromatin distribution.

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