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Bionomics and polymorphism in *Callosobruchus subinnotatus* (Coleoptera: Bruchidae)

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Abstract

Callosobruchus subinnotatus (Pic) is the major insect pest of stored bambara groundnuts, *Vigna subterranea* (L.) Verdcourt, in sub-Saharan West Africa, but little is currently known about its biology or how it may be controlled. A series of laboratory studies was performed to investigate the bionomics of and differences between two apparently different morphs of adult of each sex of this species, here termed 'active' and 'normal'. Major differences in their morphology, physiology and behaviour were identified and are described in detail for the first time. They provide clear evidence of the existence of an adult polymorphism among populations of this species, which is comparable in certain respects to that previously described for *C. maculatus* (Fabricius) and *C. chinensis* Linnaeus. Adults can be separated into the correct morph based on characteristic differences in elytral and pygidial colour and pattern. 'Normal' adults are characterized by having high fecundity, short adult life and are relatively sedentary while 'active' adults exhibit reproductive diapause (suspension of reproductive activity), are long lived, and show (at least in females) increased dispersal tendencies. These characteristics suggest adaptation of the 'active' and 'normal' morphs respectively to the different environments of field and seed stores, and the significance of the polymorphism in the life history of *C. subinnotatus* is discussed in this context. The design of any effective control regime for this bruchid needs to take account of and could potentially be based upon the existence of polymorphism in *C. subinnotatus*.

Introduction

Callosobruchus subinnotatus (Pic) (Coleoptera: Bruchidae) is the major insect pest of stored bambara groundnut, *Vigna subterranea* (L.) Verdcourt (Leguminosae). Although it has no common name, by analogy with the cowpea seed beetle, *Callosobruchus maculatus* (Fabricius), *C. subinnotatus* might be known as the bambara seed beetle. Bambara has long been considered the third most important legume as a source of protein after cowpea, *Vigna unguiculata* (L.) Walpers and groundnut, *Arachis hypogaea* L. (Leguminosae) (Sellschop, 1962) in many regions of sub-Saharan West Africa, and is now recognized as having immense potential in a wider area because of its high nutritional value (Doku *et al*., 1978) and adaptability to different environmental conditions (Padulosi quoted in Pearce, 2000). The adults lay their eggs on the pod or seed surface, and the larva then burrows into the seed, developing and feeding on the cotyledons before subsequently emerging as an adult. This causes damage in terms of seed weight loss (Amuti & Larbi, 1981), seed viability (Mbata, 1994), and market quality, with loss levels reportedly ranging from 3.7% in weight (after five months storage in Ghana (Amuti & Larbi, 1981)), to as high as 40% in Nigeria (Mbata, 1991).

The development of effective control regimes against *C. subinnotatus*, as with other pests, relies on a basic understanding of their biology and behaviour in the host

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environment, and to date this has been largely lacking for *C. subinnotatus*. Prevett (1966) observed that in Nigeria the infestations are initiated in the field before the harvest, with oviposition occurring on the driest pods which are nearest the soil surface. His observations also suggested a multivoltine habit for this species when in the storage environment, in common with many bruchid pest species, which often leads to the rapid build up of infestations over time. Development times in bambara seeds of six to seven weeks at room temperature and 40% relative humidity (r.h.) have been recorded (Prevett, 1966) and according to Mbata (1990), high humidity (>70% r.h.), and temperatures in the region of 30°C are most favourable for oviposition and development in this host. Bambara is widely thought to be the primary and preferred host, although successful development in groundnuts in the laboratory (Prevett, 1970) and cowpeas in the field (cited in Mbata, 1993) indicates that the pest status of *C. subinnotatus* may change in the future. Despite its acknowledged importance to bambara storage and the preliminary observations just cited, knowledge of *C. subinnotatus* relevant to its control is rudimentary. Much more information on the biology of this species is clearly needed before we can recommend suitable control systems appropriate to the region of production or predict how populations will respond to different control regimes.

One major aspect of bruchid biology, which has been identified among several species capable of infesting seeds both in the field and in seed stores (of which *C. subinnotatus* is one), and which could have an effect on how populations might respond to control methods is concerned with the phenomenon of adult polymorphism. The existence of two distinct forms of adult of each sex among populations of *C. maculatus* has been well documented (Utida, 1954, 1965, 1972; Caswell, 1960) and research has more recently indicated that a similar phenomenon exists in *Callosobruchus chinensis* (Linnaeus) (Silim-Nahdy *et al.*, 1999) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) (Pajni, 1986; Kaur *et al.*, 1999). In all cases, distinct differences in morphology, biology and behaviour of the two morphs have been identified and described. In *C. maculatus* which in this context has attracted the most research, probably because of its cosmopolitan importance, the two morphs of the adult have been termed 'active' and 'normal' (Caswell, 1960) because of fundamental differences in their flight tendencies (Utida, 1972; Taylor & Agbaje, 1974). The characteristics and biologies of the 'active' and 'normal' morphs of *C. maculatus* have been studied; these forms are thought to represent adaptations to the two very different environments of field and seed store respectively (Utida, 1981; Messina & Renwick, 1985). The 'normal' form is that most frequently encountered in stores but is atypical of many, if not all, non-pest bruchids which have adults more closely resembling the 'active' morph (Credland, 1990). In *C. maculatus*, the significance of the 'active' morph is thought to be related to a role in dispersal of populations from stores, because it displays increased flight tendencies compared with 'normal' adults (Utida, 1972; Taylor & Agbaje, 1974), exhibits a delay before oviposition, and is less fecund than the 'normal' form. Thus the existence of the polymorphism, and especially its role in affecting reproduction, is very important in the life cycle of the species in field conditions and therefore needs taking into account when thinking about control methods.

To date there has been no detailed description of a similar phenomenon to the polymorphism described above in *C. subinnotatus* populations, although recent research has indicated that in this species, there also exist two distinct adult forms of each sex which can arise in populations at any one time (Mbata *et al.*, 1999; J. Appleby, personal observation). Observations on adults of a population originating from northern Ghana, made prior to this study, indicated to us that a polymorphism in this species, similar in nature to that described for *C. maculatus*, did indeed exist. These preliminary observations prompted the series of studies reported here.

This paper reports investigations and descriptions of the bionomics, morphologies and dispersal abilities of adults of the two morphs of *C. subinnotatus*. In addition to providing information on the biology and characteristics of each morph, which will be useful in their identification, life table data essential to an understanding of the population dynamics of the species is provided. Production of an active, adult morph with dramatically delayed or no reproductive activity would lead to a delay if not a cessation in population growth and hence provide an effective, if unusual, means of controlling this pest species.

Materials and methods

Origin and maintenance of insects

Insects employed in these experiments were taken from a culture started with numerous individuals from infested bambara groundnuts collected in Tamale, northern Ghana, in 1997. Since then, they have been cultured on a cream-coloured cultivar of bambara groundnuts collected from the same region, which was also used in these experiments. The culture has been maintained by sub-culturing on a weekly basis, using a total of approximately 300 adults, comprising adults from at least two previous sub-cultures, on approximately 400 ml of uninfested seed (about 600 seeds). One hundred seeds of the bambara used weigh about 60 g.

Cultures were maintained in 2.5 l glass jars, covered with filter paper sealed into place with wax. All cultures and experiments were conducted in a controlled temperature and humidity room, at 27 ± 1°C, 60 ± 10% r.h., with a light dark cycle of L:D 13:11 h .

Adults did not have access either to water or food in culture or during experiments.

Source of adults for experiments

Newly emerged (< 24 h old) virgin adult males and females were used in experiments. These were obtained by isolating seeds bearing no more than two 'windows' (darkened areas of the testa through which an adult will shortly emerge) from cultures, and observing them daily for adult emergences. Preliminary observations had identified two apparently different forms of adult of each sex in laboratory cultures. Normal adults were obtained by collecting adults in the early part of the adult emergence period (28 to 36 days after establishing the culture), and active adults were obtained from seeds isolated in the latter part of the emergence period (38 to 46 days after establishing the culture).

Measurement of bionomic characteristics

Bionomic traits (adult emergence weight, oviposition period, realized female fecundity and adult longevity), were

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assessed for both adult morphs. The levels of egg mortality were measured where appropriate.

Newly emerged, virgin males and females, obtained from laboratory cultures were weighed individually in gelatine capsules on a Sartorius Micro balance (± 0.001 mg), and then paired in glass tubes containing ten bambara beans and lined with coarse grade emery paper; coarse grade emery paper deters oviposition on the glass tube itself. Thirty eight pairs of normal individuals were established and the same number of pairs of active individuals. Every 24 (± 2) h, the number of eggs per seed was counted and the seeds in each tube replaced with pristine ones, until female death. Egg-laden seeds were retained for 12 days before recording whether or not successful larval penetration of the seed testa had occurred, shown by the presence of white frass in the egg case. 'Egg hatching' is here equated with successful penetration of the seed testa by a first instar larva which is practically, if not physiologically, appropriate. The time of death of each adult beetle was recorded.

Statistically significant differences in the bionomic characteristics of the morphs of each sex were identified using either parametric or, where necessary, non-parametric one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls (SNK) multiple comparisons tests to identify individual differences. Tests (derived from Dunn, 1964) utilizing rank sums were used after non-parametric ANOVA with unequal sample sizes (Zar, 1996)

Description of adult morphs

To identify and describe the differences in external morphology, live adults of both sexes and both morphs were examined. Differences in elytral patterning and body shape between the two morphs were then used to assign other adults taken at random from cultures as active or normal, male or female. A sample of 30 active males, 30 active females, 30 normal males and 30 normal females was collected. Morphological differences among these adults were quantified by measurement of a total of 22 external body characters (table 1) using a calibrated ocular micrometer.

One-way ANOVA was used to determine the significance of differences among adults for each of the 22 measured characters. Discrimination among adults of the four groups based on these characters was assessed using Multiple Discriminant Analysis.

Abdominal dissections were then performed on each adult under saline, and the morphology of the reproductive system examined in order to confirm the morph and sex of each individual.

Dispersal behaviour of the adult morphs

Preliminary data on dispersal behaviour of the morphs were obtained in two ways. First, in cultures containing both active and normal morphs, the proportion of adults of each form to be found in the jar in total, and the proportion which had dispersed from the seeds to rest on the filter paper in the neck of the jar were counted. The second indicator was simply to release adults of known form from a height of 1.5 m and record whether they flew before plummeting directly to the ground; at least 100 replicates with each morph of each sex were undertaken.

Results

Measurement of bionomic characteristics

Emergence weights

The mean emergence weight of adult *C. subinnotatus* differed between the sexes and also between the two forms, normal and active ($F_{3,148} = 58.99$; $P < 0.001$) (fig. 1). Males were significantly heavier than females, by approximately 9% among normal adults and by about 15% among active adults. Differences were also apparent between the weights of active compared with normal adults of each sex, with active males being about 13% lighter and active females being about 19% lighter than normal adults of the same sex.

Oviposition

Normal females began oviposition within 24 h of their emergence and subsequent pairing with a male (fig. 2), but did not reach maximum egg laying capacity until one or two days after pairing. Thereafter the mean number of eggs laid in any 24 h period declined steadily, and by 12 days after pairing only 2 out of 38 females were still ovipositing. The mean realized fecundity of normal females was 107 eggs ± 5 (standard error) (median = 111), and egg mortality levels among the eggs laid by a single female averaged 16% (range 2–38%).

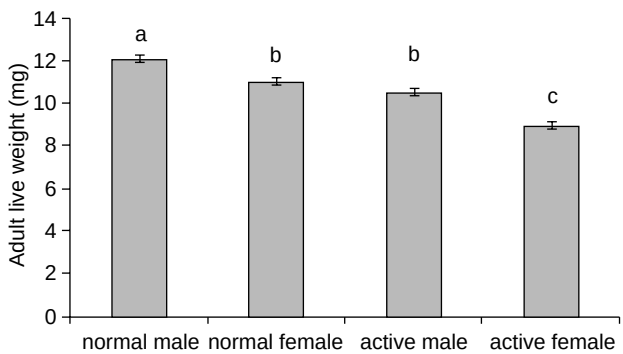

Fig. 1. Mean live adult weights (mg) for both morphs of each sex of *Callosobruchus subinnotatus* determined within 24 h of emergence. Bars represent standard errors. (Bars bearing different letters are significantly different from each other, $P < 0.05$).

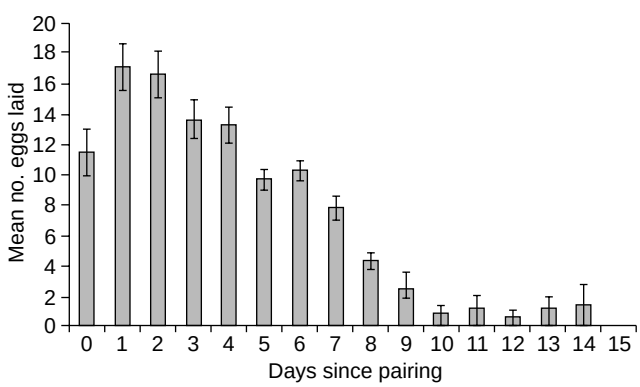

Fig. 2. Daily oviposition by normal females of *Callosobruchus subinnotatus* when offered ten pristine bambara seeds every 24 h from emergence until death. Bars represent standard errors.

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Table 1. Means (± standard errors) for each of the 22 characters measured in adults of the four groups; active female, normal female, active male and normal male of *Callosobruchus subinnotatus*.

Character	Normal male	Active male	Normal female	Active female	*P*
Max. body length (dorsal view)	5.55^{ab} ± 0.05	5.28^{a} ± 0.10	5.62^{b} ± 0.06	5.41^{a} ± 0.05	0.001
Max. body width (dorsal view)	1.76^{b} ± 0.01	1.72^{ab} ± 0.02	1.69^{a} ± 0.02	1.71^{ab} ± 0.04	0.020
Head length	1.49^{b} ± 0.01	1.48^{b} ± 0.01	1.45^{ab} ± 0.01	1.42^{a} ± 0.02	0.000
Head width (at eyes)	1.20^{b} ± 0.01	1.20^{b} ± 0.01	1.045^{a} ± 0.01	1.053^{a} ± 0.01	0.000
Vertex length	0.56^{a} ± 0.01	0.60^{b} ± 0.01	0.54^{a} ± 0.01	0.56^{a} ± 0.01	0.000
Vertex width (at anterior edge of eyes)	0.19^{b} ± 0.01	0.18^{a} ± 0.00	0.20^{ab} ± 0.01	0.20^{b} ± 0.01	0.050
Antennal length	2.51^{b} ± 0.02	2.50^{b} ± 0.02	2.05^{a} ± 0.01	1.97^{a} ± 0.02	0.000
Length of apical antennal segment	0.32^{b} ± 0.01	0.33^{b} ± 0.01	0.26^{a} ± 0.00	0.25^{a} ± 0.01	0.000
Pronotal length	1.17^{b} ± 0.01	1.16^{ab} ± 0.01	1.18^{b} ± 0.01	1.15^{a} ± 0.02	0.003
Pronotal width	1.68^{b} ± 0.01	1.67^{b} ± 0.02	1.66^{b} ± 0.01	1.62^{a} ± 0.02	0.002
Abdominal length (ventral view)	2.27^{a} ± 0.04	2.22^{a} ± 0.05	2.57^{b} ± 0.03	2.26^{a} ± 0.03	0.000
Max. abdominal width	2.08^{b} ± 0.02	2.04^{b} ± 0.02	2.03^{b} ± 0.02	1.96^{a} ± 0.02	0.000
Pygidial length (dorsal view)	0.58^{ab} ± 0.03	0.50^{a} ± 0.03	0.76^{c} ± 0.03	0.70^{bc} ± 0.04	0.000
Max. pygidial width (dorsal view)	1.64° ± 0.01	1.59^{bc} ± 0.01	1.58^{ab} ± 0.01	1.48^{a} ± 0.04	0.000
Femur length	1.63^{b} ± 0.01	1.60^{ab} ± 0.02	1.55^{a} ± 0.02	1.57^{ab} ± 0.01	0.006
Max. femur width	0.55^{a} ± 0.01	0.59^{b} ± 0.01	0.57^{ab} ± 0.01	0.58^{ab} ± 0.01	0.013
Tibia length	1.10^{b} ± 0.02	1.06^{ab} ± 0.01	1.09^{b} ± 0.01	1.00^{a} ± 0.02	0.000
Max. tibia width	0.55^{a} ± 0.01	0.59^{b} ± 0.01	0.57^{ab} ± 0.01	0.58^{ab} ± 0.01	0.013
Max. elytral length	2.81^{c} ± 0.02	2.73^{b} ± 0.02	2.60^{a} ± 0.02	2.58^{a} ± 0.02	0.000
Max. elytral width	1.24^{b} ± 0.01	1.22^{b} ± 0.01	1.17^{a} ± 0.01	1.18^{a} ± 0.01	0.000
Max. hindwing length	5.38^{c} ± 0.05	5.30^{c} ± 0.05	4.62^{a} ± 0.04	4.92^{b} ± 0.03	0.000
Max. hindwing width	1.93^{c} ± 0.03	1.94^{c} ± 0.02	1.62^{a} ± 0.02	1.76^{b} ± 0.03	0.000

All measurements are in millimetres. The significance of differences among the four groups for each character is indicated by the *P* value obtained by ANOVA; values of *P* are correct to three decimal places. Means in the same row bearing different letters are significantly different from one another.

In contrast, out of the same number of active females sampled, all but one female failed to deposit even a single egg during their entire lifetime, despite constant access to both a potential mate and a suitable oviposition substrate (bambara seeds). Furthermore, none of the nine eggs laid by the single laying female hatched, suggesting that even these eggs were infertile. The mean realized fecundity of active females was therefore only 0.24 eggs (median = 0) and egg mortality in the laboratory was 100%. Comparison of the fecundity of the normal and active females, revealed an obvious and profound difference.

Adult longevity

Despite their lower emergence weights (fig. 1), active adults lived, on average, for more than twice as long as normal adults (average longevity of 23 days for active males and females, compared with 11 days for normal males and females), and this was without any form of sustenance during the experiment (fig. 3). Analysis using Kruskal-Wallis one-way ANOVA showed significant differences in the longevities of adults belonging to the four groups (χ^2= 62.97, $P < 0.001$), and multiple comparisons confirmed that these differences were in the longevities of active compared with normal adults, and were independent of sex.

Description of adult morphs

External morphology

The most obvious morphological differences between active and normal adults are the shape of the body, and the

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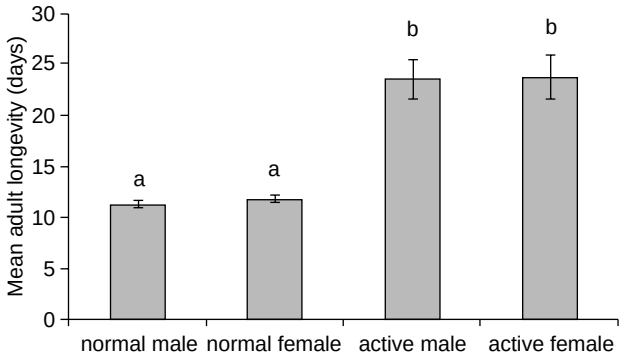

Fig. 3. Mean adult longevity (days) for both morphs of each sex of *Callosobruchus subinnotatus* when maintained in pairs and offered ten pristine bambara seeds as oviposition sites every 24 h from emergence until death. Bars represent standard errors. (Bars bearing different letters are significantly different from each other, $P < 0.05$).

colour and patterning of the elytra and pygidium. These differences are most pronounced in females of the two groups.

At emergence, in the normal female the abdomen extends well beyond the end of the elytra, and is fat and rounded posteriorly (fig. 4a). The abdomen of the active female is narrower and more pointed posteriorly (fig. 4b), and is flatter when viewed from the side.

Differences in the elytral and pygidial pattern of the two female morphs appear to be attributable to differences in the amount and distribution of pubescence (fig. 4). In both morphs the ground colour of the elytra is black or nearly black, and the pubescence is white. Typically, in the normal female, the pubescence forms a distinct 'cross' pattern across the two elytra, consisting of discrete patches of pubescence against a black background of bare cuticle. There is also a distinctive white stripe of pubescence down the dorsal midline of the pygidium. In the active female, however, the pubescence on the elytra is much more diffuse, excepting two lateral regions, which gives them a grey appearance. The pubescence on the pygidium is more abundant, but at the same time more diffuse, with no conspicuous central white stripe.

In males, there is no obvious difference in body shape between the two morphs, and differences in the elytral patterning are much more subtle and variable. The main difference appears to be in the colour of the cuticle itself rather than the distribution of pubescence. In normal males the cuticle is usually tan in colour across the whole of the elytra, while in active males there are typically several patches of dark brown cuticle, varying to the extent that the whole of the elytra may appear very dark brown. Thus active males tend to look much darker than normal males.

Morphometric analyses

The aim of these analyses was to identify and quantify measurable differences in the external morphologies of the two morphs of each sex, and to determine whether these differences could be used as a tool for the simple, unambiguous classification of adults into the correct morph.

The 22 characters which were measured are listed in table 1. The mean and associated standard error for each character is shown against the sex and morph of adults, assigned to each group based on the differences in body shape and elytral and pygidial colour and pattern described above. The significance of differences among the means, and which of the values differ from each other for each character are also indicated.

Comparisons between the male and female adult groups of either the normal and/or the active morph, showed significant differences in 18 of the 22 characters, and these differences were consistent with some of the qualitative descriptions of males and females given by Southgate (1958); males were found to have significantly longer antennae than females, particularly in the length of the last antennal segment. Also, the significantly wider head of males is probably due to them having more protuberant eyes than females (Southgate, 1958).

Differences between the two morphs were identified in eight characters among females, and four characters among males. The most biologically significant of these was the larger size (both in width and length) of the hindwing of active compared with normal females, despite a significantly

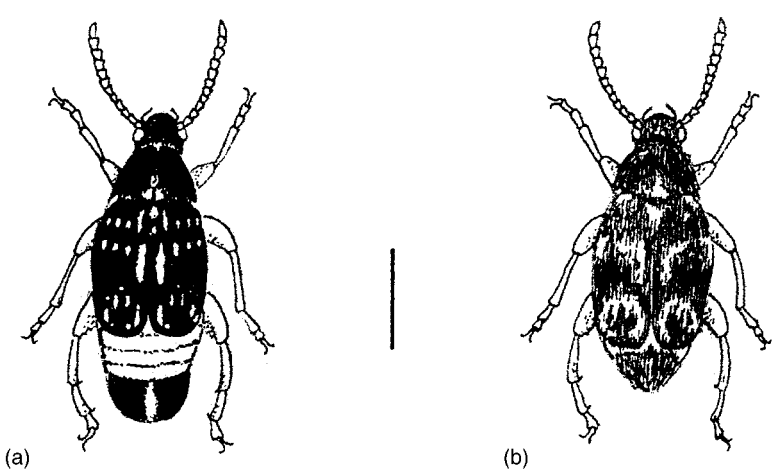

Fig. 4. Typical normal female (a) and active female (b) of *Callosobruchus subinnotatus* within 24 h of emergence, showing the characteristic patterns of pubescence on the elytra and pygidium. Scale bar is 2 mm.

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shorter body length and lower body weight, which might be an indicator of increased flight capacity. Despite these differences however, no character could be used in isolation to discriminate between active and normal adults, regardless of sex.

The multiple discriminant analyses showed how successfully adults of the four groups could be discriminated from each other using some combination of the 22 characters (variables). Table 2 shows the percentage of variance in the grouping variable (adult morph/sex), which is explained by each of the three computed discriminant functions. The first function alone explained over 74% of the variation, and was expected to be the most significant in contributing to the classification of individuals. In fact, all three functions contributed significantly to classifying individuals into the four groups, as shown in table 3. Those characters that were most highly correlated with the first two discriminant functions included antennal length and hindwing lengths and widths, indicating that these were among the most discriminating characters, as predicted from the ANOVA conducted previously.

Graphical representation of the discrimination of each individual case among the four groups by the first two functions is shown in fig. 5. The first function (represented on the horizontal axis), clearly separated two discrete clusters; the first cluster on the left of the graph constitutes the two male groups, represented by letters 'c' and 'd', and the second cluster on the right of the graph, constitutes the two female groups, represented by letters 'a' and 'b'. Both of the two functions served to separate these two main clusters further, seen most clearly in the separation of the two female groups into active females represented by letters 'a', and normal females, represented by letters 'b', and to a lesser extent in the separation of the two male groups by letters 'c' and 'd'.

To conclude the analyses, the classification of all the individual insects according to the computed discriminant functions was examined to see how well this conformed with the four groups of adults to which individuals had been assigned previously, based purely on the qualitative differences in their elytra and body shapes. This was very successful, with overall 93.3% of cases being classified into the correct adult group. The actual number and percentages attributed to each group are given in table 4. The few adults that were mis-classified were almost all active males identified as normal, or vice versa. One active female was mistakenly classified as an active male.

Table 2. Eigenvalues and percentages of variance explained by the three functions of the discriminant analysis.

	Eigenvalue	% Variance	Cumulative % variance
Function 1	8.41	74.4	74.4
Function 2	2.13	18.8	93.2
Function 3	0.77	6.8	100.0

Table 3. The contribution of the three discriminant functions to the classification of individual adults of *Callosobruchus subinnotatus* into the four groups.

After function	Wilks' lambda	Chi-squared	Degrees of freedom	*P*
1	0.019	418.85	66	0.000
2	0.181	181.24	42	0.000
3	0.566	60.30	20	0.000

Values of *P* are correct to three decimal places.

Table 4. Classification of adults of the four groups of *Callosobruchus subinnotatus* according to the discriminant functions.

Actual/predicted group	Group 1	Group 2	Group 3	Group 4
Active female	29 (96.7)	–	1 (3.3)	–
Normal female	–	30 (100)	–	–
Active male	–	–	26 (86.7)	4 (13.3)
Normal male	–	–	3 (10.0)	27 (90.0)

Predicted group memberships for adults observationally determined as belonging to one of four groups (active female, normal female, active male, normal male), are indicated by actual numbers (percentage values in parentheses).

Reproductive system

Abdominal dissections of adults belonging to the four groups (normal, active, male and female), revealed major differences in the morphologies of their reproductive systems at emergence (fig. 6). In the two female groups, the main differences are in the size and structure of the ovarioles (fig. 6a). The normal female has a pair of large, well developed ovaries with developing oocytes of different sizes and at different stages of maturity clearly visible along the length of the vitellarium. In active females however, the reproductive system is immature. The ovarioles are very small and are limited to the germarium, with no vitellogenic oocytes visible, suggesting the absence of yolk production or deposition in these individuals. In the two male groups (fig. 6b), the differences are in the size of the testes and in the

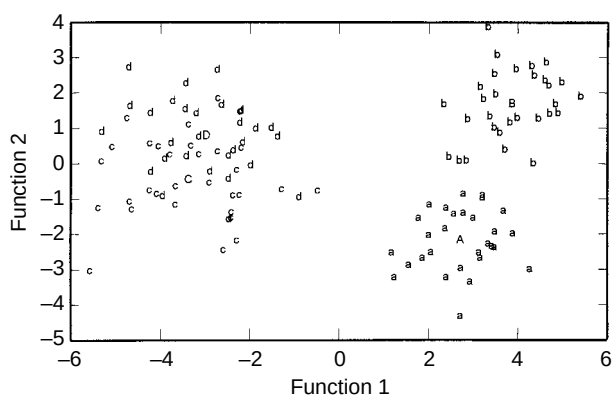

Fig. 5. Disposition of individuals of the two adult morphs of each sex of *Callosobruchus subinnotatus* according to the first two discriminant functions, obtained from a canonical discriminant analysis using 22 characters measured in adults of four different types (active females, normal females, active males and normal males, represented by letters a,b,c and d respectively). Group centroids are indicated by capital letters.

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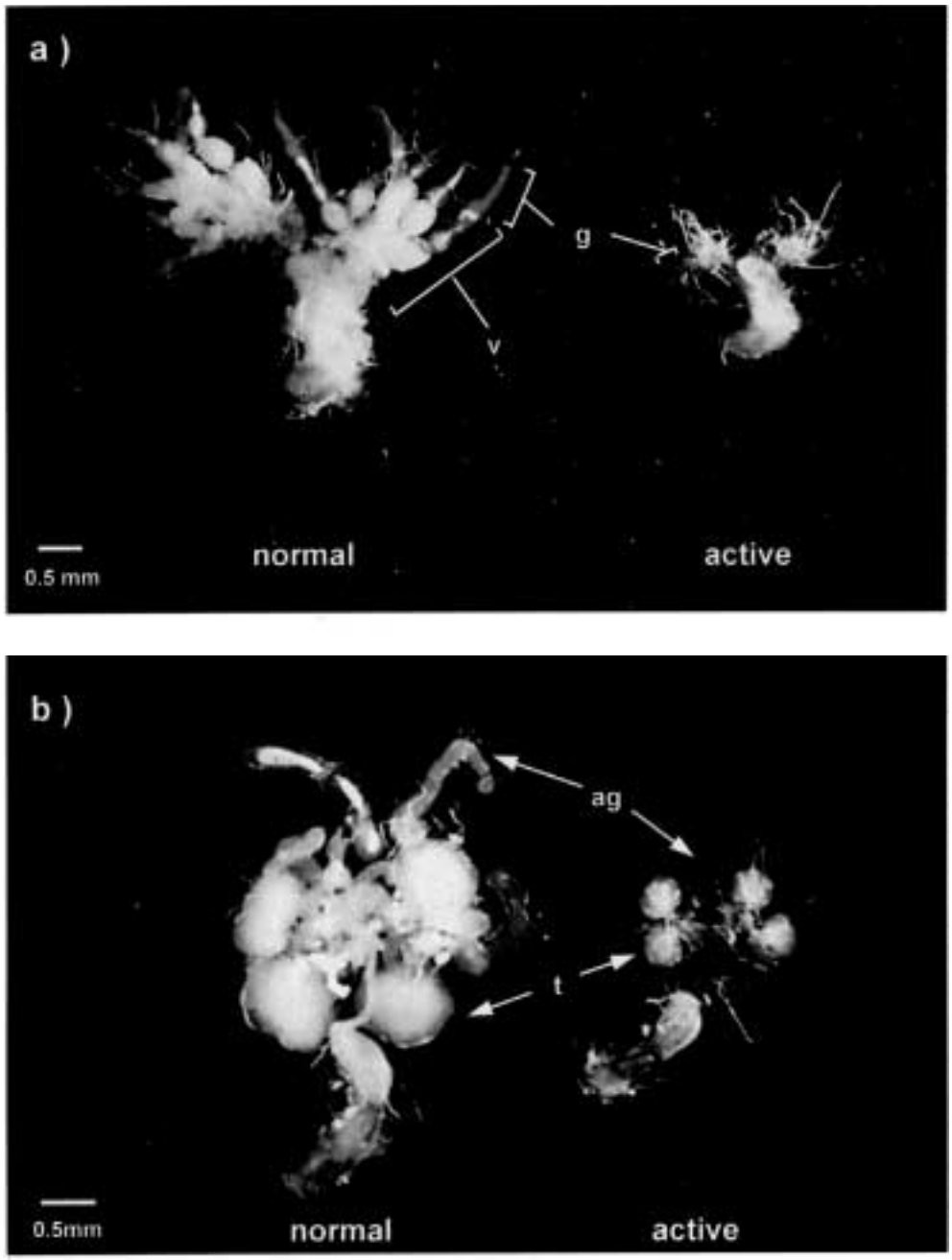

Fig. 6. Reproductive systems of (a) the normal and active female, and (b) the normal and active male of *Callosobruchus subinnotatus* shortly after emergence. v, vitellarium; g, germarium; ag, accessory gland; t, testis.

morphology of the accessory glands. Normal males are characterized by having large, well developed testes, and dense secretions (seminal fluid) are visible in the median accessory glands. In active males, however, the reproductive system is immature, with much smaller testes, and accessory glands completely lacking in secretions.

In addition to the above, adults of both sexes of the active morph were observed to have an abundance of fat body in the abdominal cavity, considerably in excess of that in normal adults.

Examination of the reproductive system of every individual subjected to morphometric analysis concurred with the outcome of the discriminant analysis (table 4) including those which had initially been mis-classified on the basis of their external morphology.

Dispersal behaviour of adult morphs

The proportions of active adults amongst those adults which had dispersed from the bean heap to the neck of the

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culture jar were very high, being 87.8% (where 92% of females and 82.5% of males were of the active morph), 90% (where 100% of females and 83.3% of males were active) and 77% (where 95% of females and 68% of males were active), in three consecutive laboratory cultures from which adult counts were made. These high proportions were not due to a predominance of actives in each population as a whole, since active adults made up only 43%, 3% and 30% respectively of the entire adult population in each culture at the time of observation. This would suggest that active adults have a higher tendency to disperse from bean heaps than do normal adults.

When released from a height of 1.5 m, 58% of active females flew while only 20% of normal females flew, suggesting that active females are more likely to fly than normal females. In males, surprisingly, it was the other way round, with normal males flying 91% of the time, while active males flew only 64% of the time. Dispersal may, of course, include walking as well as flying.

Discussion

Callosobruchus subinnotatus has been subject to little research to date, despite being a bruchid known to infest and cause losses to stored bambara seeds which are of considerable local importance for both economic and nutritional reasons. There have been only a few studies, predominantly by Mbata (Mbata, 1990, 1993; Mbata *et al*., 1997), which have attempted to describe significant features of its biology, although it is widely recognized that such knowledge would enhance, and is indeed a prerequisite for, the development of any effective control regimes. Furthermore, our knowledge of bruchids is limited to studies of very few species, and most of these, such as *C. maculatus*, *C. chinensis* and *Z. subfasciatus*, are those which are of cosmopolitan importance as pests of primary food crops of international importance. Each additional species studied therefore extends our knowledge of the bruchid family and enables us to make more generalized statements in which we have greater confidence.

This study revealed that *C. subinnotatus* is fecund, with individuals of the strain employed here typically laying approximately 107 eggs on its preferred host, bambara groundnut. This is only very slightly higher than that recorded by Mbata (1990), under comparable environmental conditions using a different strain of beetles, from near Nsukka in southern Nigeria, which had been offered six seeds daily. Also in agreement with Mbata (1990), the present study found that most eggs were laid in the first days of adult life and adults die after about 11 days (at 27°C and 60% r.h.). However, the present study has shown that such statements are only true of normal adults. It is now entirely clear that this species, together with some other members of the Bruchidae, including *C. maculatus* (Utida, 1954; Caswell, 1960), *C. chinensis* (Silim-Nahdy *et al*., 1999), and *Z. subfasciatus* (Kaur *et al*., 1999), is polymorphic with adults of both sexes occurring in two distinct forms, differing in morphology, physiology and in behaviour.

Southgate *et al*. (1957) and later Caswell (1960) were the first to use the terms active and normal to describe adults of the two morphs of *C. maculatus*, using these terms as opposed to 'flight' and 'non-flight' (Utida, 1954) because, although the active form was observed to fly more frequently than the normal form, both morphs in fact have the capacity both to walk and to fly. Since similar observations have now been made for the two morphs of *C. subinnotatus*, it seems preferable to refer to them as 'active' and 'normal'.

The abdominal dissections and measurements of fecundity of individuals clearly showed that the reproductive systems of active male and female *C. subinnotatus* are non-functional at emergence, and these adults will not mate or begin oviposition when reared and maintained under the stated conditions. This suspension of reproductive activity was found to be correlated with numerous physiological features, including the absence of vitellogenic oocytes in the ovarioles of active females, the small testes and absence of secretions in the accessory glands of active males, and the increased amount of fat body in active adults; together these characteristics indicate that these adults are in reproductive diapause (suspension of reproductive activity (Danilevskii, 1965)). A suspension of reproductive activity is correlated with almost identical physiological differences in the two other bruchids, of different genera, known to exhibit reproductive diapause, *Bruchidius atrolineatus* Pic (Glitho & Huignard, 1990; Germain *et al*., 1985), and *Bruchus rufimanus* Boheman (Tran & Huignard, 1992). Unlike *C. subinnotatus*, neither of these species is known to exhibit adult polymorphism and so in *Bruchidius atrolineatus* and *Bruchus rufimanus* the physiological differences are the only effective way of identifying diapausing and non-diapausing individuals as such.

In *Bruchidius atrolineatus*, and most other diapausing species, specific cues have been identified which are necessary to terminate diapause and stimulate reproductive activity (Germain *et al*., 1985; Monge & Germain, 1988; Lenga *et al*., 1993). In *C. subinnotatus* it appears that, at least under the environmental conditions employed here, the reproductive systems of active males and females do not mature within 20 days of their emergence, even when maintained with reproductively active mates and host seeds (J. Appleby, unpublished observations). This is in contrast with *C. maculatus*, where eggs do begin to develop in the ovarioles of active females, followed by copulation and oviposition, some three or four days after emergence (Utida, 1972), and it suggests that for *C. subinnotatus* a specific cue may be necessary to stimulate reproductive maturation. These characteristics of the active morph of *C. subinnotatus*, suspension of reproductive activity (reproductive diapause) and increased longevity, indicate that like diapausing *B. atrolineatus*, it may represent a means of population survival during periods when host seed is absent from the field and/or seed store. If so, the active morph may therefore actually represent the normal condition of univoltine bruchids which do not have access to large stores of hosts but rather depend upon their seasonal availability. Dispersal from these hosts will then be essential and the adult beetles must then persist in the environment until the next generation of seeds becomes available. The normal morph would then represent that normally encountered in seed stores, rather than being the ancestral form.

It is shown that *C. subinnotatus* adults of the normal form are heavier than adults of the active form of the same sex, and also that males are heavier than females. There is evidence (J. Appleby, unpublished observations), that mean adult weight declines when seeds are infested with more than one larva, although this did not account for the weight

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differences here since adult insects were only taken from seeds known to contain no more than four developing insects at which density there is no evidence of declining weight (J. Appleby, unpublished observations).

The differences in colour and patterning of the elytra and pygidia of the two morphs are characteristic, but there is considerable variation around the basic plan described above, especially between the two male morphs. Colour and pubescence differences have also been described as characteristic of the adult morphs of *C. maculatus* (Utida, 1972), *C. chinensis* (Silim-Nahdy *et al.*, 1999), and *Z. subfasciatus* (Kaur *et al.*, 1999), where there is also a certain amount of variation. The precise nature of the elytral differences are species specific, but it is interesting to note that the pygidial differences observed between the two female morphs of *C. subinnotatus* are very similar to those observed in both *C. maculatus* and *Z. subfasciatus*. Other differences, revealed in the morphometric analyses, are also largely consistent with some of the those identified between the adult morphs of *C. maculatus* and *C. chinensis* (Yasui, cited in Utida, 1972; Silim-Nahdy *et al.*, 1999), most notably in parameters to do with wing size, which is consistently relatively larger in the active females of all three species, and correlates with their increased flight tendencies.

The multiple discriminant analysis reveals quantitatively what can be deduced from the colour and behavioural differences. There are clear distinctions among the four groups of adults (male or female, active or normal), when the morphological characters are taken in combination. Unfortunately no single character allows either sex to be allotted with absolute confidence to the active or normal group, simply because there is always some overlap between them. However, knowing that these differences are correlated with the differences in reproductive morphology and the differences in elytral and pygidial colour and pattern, it is proposed that the latter differences are used as the most effective means of morph classification. Where it is appropriate to do so, adults can be dissected and the state of the reproductive system, according to aforementioned criteria, used as an alternative method of identifying the state of individuals.

When the morphological differences are taken together with the differences in the bionomics of the two morphs and their reproductive physiologies, it appears that there is a suite of characters which support the notion of the active form being better adapted to dispersal from seed stores and survival in the field situation, while the normal morph is better adapted to life within the seed store. Thus, the active female has larger wings, flies more frequently, exhibits reproductive diapause associated with zero early fecundity and is long-lived, while the normal female is relatively sedentary, short lived and highly fecund. Utida (1972) has suggested that the normal and active forms of *C. maculatus* may similarly be adapted to life in stores and dispersal respectively. If the active morph is indeed the ancestral morph, as is most likely since the field environment with seasonal availability of hosts would have been available before the host abundant storage environment, then these findings add further evidence to the hypothesis that pest species have evolved to produce a highly fecund, short-lived morph (here termed the normal morph), which can rapidly utilize the highly concentrated hosts which man describes as a store. It thereby appears that *C. subinnotatus* has been able to become a significant pest of bambara, and possibly other legumes.

The important remaining questions are 'What factors influence which of the two morphs emerges from a seed?', and 'What stimulus is required to enable the 'active' form to become reproductively active?' Information derived from studies on other bruchids may provide clues to the answers to these questions. For example, in *C. maculatus* there is certainly a genetic component involved in production of the active form (Messina, 1987) but the genotype-environment interaction is critical. Temperature, seed moisture content, photoperiod and population density have all been seen to initiate reproductive diapause and/or production of morphs in other insects (Denlinger, 1986), including bruchids (e.g. Lenga *et al.*, 1991). It has to be assumed that the active morphs become sexually active at some time after their production. In *Bruchidius atrolineatus* and *Bruchus rufimanus*, both temperature and photoperiod play parts in initiating gonad development and sexual activity after periods of reproductive diapause but in both cases adults only become responsive after a protracted period of sexual inactivity (Lenga *et al.*, 1993; Tran & Huignard, 1992). The cues for active morph induction and diapause termination in *C. subinnotatus* are currently the subject of further investigation.

If the cues which promote the production of active morphs and hence reproductive diapause can be identified and the storage environment could be manipulated accordingly, this may lead to effective, environmentally-friendly forms of pest control without recourse to the use of chemicals.

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