

EXPERIMENTAL ANALYSIS OF ADHESION OF
CHRYSOLINA POLITA (CHRYSOMELIDAE: COLEOPTERA)
ON A VARIETY OF SURFACES

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(Received 12 December 1979)

SUMMARY

1. Pulling forces of *Chrysolina polita* (L.) on glass, perspex and cloth have been recorded and are plotted against body weight. The additional weight of eggs in many of the females is probably the main reason that, in females, pulling forces do not increase significantly with body weight, whereas those of males do.

2. Pulling forces on glass and perspex are significantly correlated but, in general, neither are significantly correlated with those on cloth. This is because traction on glass and perspex is achieved by the adhesive setae, and on cloth by the tarsal claws.

3. Counts of the adhesive setae in 14 individuals indicate that numbers of climbing setae in females and males and females (combined), male setae in males, and the total number of adhesive setae in males, all show a significant increase with body weight.

4. Pulling force increases with the total number of adhesive setae in females, and males and females (combined). Multiple regression analysis confirms the view that pulling force is a function of the number of adhesive setae.

5. Hooks, suction, electrostatic forces and seizure are largely discredited, and molecular adhesion between the setae and the substratum, and possibly the cohesive forces and surface tension of a thin fluid layer, are confirmed as the most likely modes of adhesion on smooth surfaces in *C. polita* and most beetles.

6. It is proposed that the main forces of detachment acting on a leaf beetle are probably drag and the whiplash effect of the leaves and branches. Estimates for the wind speeds required to remove beetles from glass and cloth by drag forces alone are calculated.

INTRODUCTION

The phenomenon of the ability of some insects and other animals to adhere to smooth surfaces has always fascinated naturalists. The fundamental issue to the early microscopists was how the 'fly' could hold on to glass against the force of gravity. In the mid-nineteenth century there was considerable discussion on this subject. Indeed, today well over a hundred years later, there is still controversy about the actual

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mode(s) of adhesion employed. The major problem in understanding the derivation of the adhesive force has been the dearth of accurate information on the detailed structure of the adhesive setae. Stork & Evans (1976) used the SEM to reveal the diversity of structure in coleopteran adhesive setae which had been hinted at by West (1862), Simmermacher (1884), Dahl (1884) and Jeannel (1941). Recently, Stork (1980) has studied the morphology of adhesive setae in the Coleoptera more fully, suggesting reasons for their diversity.

Apart from Plateau (1872), who measured the pulling force in a few caraboids, no one has made a serious attempt to estimate the adhesive forces involved in traction of adhesive setae on smooth surfaces. This paper first examines 'pulling force' attained on several surfaces in *Chrysolina polita* (L.) in relation to body weight and sex, and secondly, investigates the mode(s) of adhesion involved in the use of adhesive setae.


MATERIALS AND METHODS

(i) *General experiment*

A George Washington Ltd 50 g force transducer Type D1 was coupled to a George Washington Ltd ink-writing oscillograph MD/2. The transducer was clamped to a Sonnenblik screw stand so that the optimum sensitivity to pulling was in the vertical plane. A sheet of glass, previously cleaned with acetone, was clamped behind the force transducer. Sixty-two specimens of *Chrysolina polita* (L.) (Chrysomelidae; Coleoptera) were individually weighed and a small loop of fine electrical wire attached to each of their elytra. This was accomplished by anaesthetizing them with carbon dioxide and gluing on the wire loops with a small amount of a mixture comprising 7 parts beeswax to 4 parts pale amber resin (BDH Chemicals Ltd.). The beetles were then reweighed and left in a cold room (approx. 15 °C) with an adequate supply of their food source (water mint, *Mentha aquatica* L.). Before being attached to the force transducer the beetles were allowed to warm up to room temperature (approx. 20–25 °C) for about 30 min. The beetles were hooked on to the force transducer and 10 min recordings were taken on a suitable attenuation for each beetle walking on the glass substratum. Similar recordings were taken on perspex (also previously cleaned with acetone) and rough-woven linen cloth (the latter was stretched over a piece of glass). The force transducer was calibrated before and after each trial. A gap of several hours was left between recordings for an individual beetle on the different substrates. Recordings other than those made when the beetle was pulling in the direction of optimum sensitivity of the force transducer (i.e. vertically) were discarded.

Chrysolina polita (L.) is a moderately large (6.5–8.5 mm) 'leaf beetle' found in waterside situations throughout much of the Palaearctic region. The larval host-plants are species of *Mentha* and other Labiatae. The adult beetles climb these and a variety of other waterside plants.

(ii) *Number of adhesive setae*

When the recordings had been taken for each beetle it was placed in a dry atmosphere to die. The tarsi of 20 individuals were mounted on SEM stubs and gold coated with an Edwards S150 sputter coater. The specimens were examined with a Cambridge S140 SEM and counts of the numbers of adhesive setae were made from electro-

(iii) Removal of tarsal claws

The pulling forces of 15 specimens of *C. polita* were recorded on glass, perspex and cloth. The beetles were anaesthetized with carbon dioxide and their tarsal claws amputated distal to the third and fourth tarsomeres, using micro-scissors. The beetles were allowed several hours to recover before their pulling forces were recorded again on the same substrates. The results from the two treatments were compared using multiple regression analysis.

(iv) Suction

A smaller but identical version of the pulling apparatus (with the exception of the pen-recorder) was placed under an inverted bell jar. A beetle was attached to the force transducer and recordings were taken using clean glass as the substratum. After several minutes the pressure of the atmosphere in the bell jar was slowly reduced in stages of 100 mm of Hg down by a maximum of 600 mm of Hg reduced pressure, using a vacuum pump. The pressure was held for about a minute at each stage. Ten specimens (five of each sex) of *C. polita* were used in this experiment.

(v) Reduction of electrostatic forces

Pulling force recordings were taken for ten individuals of *C. polita* on glass, perspex and cloth. After each beetle had started to pull near the maximum of its ability on each substrate a Zerostat Antistatic Gun was held 12 in. from the beetle and slowly fired (as described in the instructions for the gun) four times in succession. The gun, when fired, emits ions from a piezo-electric crystal neutralizing the electrostatic forces. The beetles' pulling forces were also recorded on a sheet of glass that had been coated with a fine layer of gold (using an Edwards S150 sputter coater). The gold-coated glass was earthed by a length of electrical wire attached at the other end to the screw stand. The pulling forces from both sets of experiments were analysed by multiple regression.

(vi) Clean and dirty glass

Pulling forces were recorded for *C. polita* on a sheet of glass that had been handled and not cleaned before use. The sheet of glass was then thoroughly cleaned with acetone and absolute alcohol, and pulling force recordings were taken again for the same beetles. The results were analysed by multiple regression.

(vii) Dry atmosphere

The whole pulling apparatus, with the exception of the pen recorder, was placed in an air-tight Bassaire DB/H glove box, A number of beetles in closed containers and an open-topped beaker containing about 50–100 g of phosphorus pentoxide (to dry the atmosphere of the glove box) powder were also placed in the glove box. The apparatus was then left for 24 h to allow the phosphorus pentoxide to absorb the water vapour in the air. Pulling force recordings for individuals of *C. polita* were made on glass that had been cleaned in acetone and alcohol before being placed in the glove box. Before being placed on the substrate the beetles were allowed to walk on filter paper to remove some of the fluid (if present) from their tarsi. These pulling-force recordings

have been compared by multiple regression analysis with normal pulling force recordings of other individuals taken in the same week.

In this study the maximum recorded force a beetle achieves on a particular substratum is called its *pulling force*. The pulling force for each beetle was found by calibrating the oscillograph recordings with weights and adding to the maximum recorded force, the weight of the beetle, its individual hook, and that of the hook on the force transducer.

RESULTS

(i) *General experiment*

It is fortunate that the beetles used in these experiments nearly always walked vertically up the substrates and therefore pulled in the direction of optimum sensitivity of the force transducer. In most cases the beetles achieved a maximum pulling force that was very little increased after the 10 min recording periods used in this experiment. Fig. 1 shows several photographs of individuals attached to the force transducer whilst adhering to glass. In Fig. 1(c) an unrestrained beetle is walking up the sheet of glass and its tarsi are unevenly spaced with respect to each other and to the rest of the body. In contrast, the beetles in Fig. 1(a) and (b) are pulling against the force transducer and the tarsi are more aligned and closer together. It is noticeable in both these photographs that only one tarsus is moving (slightly out of focus) at any one time. In Fig. 1(d) the beetle has been pulled down the glass (by winding down the screw stand to which the force transducer is attached) and the adhering tarsi are being dragged behind the descending beetle. An example of part of a recorded trace of a beetle pulling (as in Fig. 1a, b) is shown in Fig. 2. After calibration of the force transducer with weights the maximum pull on this trace is equivalent to 33.8 mN.

The pulling forces of 62 specimens of *C. polita* on glass, perspex and cloth are plotted against body weight in Figs. 3–5 and mean values are shown in Table 1. Although the mean body weight for females is almost twice that of the males, there appears to be little difference in their mean pulling forces on all three surfaces. Only the regression coefficients (S) for the pulling forces of the males against body weight on all three surfaces, and that on cloth for the males and females (combined), are significantly different from zero. Therefore, only the four regression lines are shown in Figs. 3–5. The correlation coefficients (R) for males ($R = 0.83$), females ($R = 0.68$) and males and females (combined) ($R = 0.76$) for the pulling forces on glass and perspex are all significantly different from zero at the 0.1% level. These contrast with the correlation coefficients of all the groups for pulling forces on glass and cloth, and perspex and cloth, all of which (except that of the males and females (combined) pulling forces on glass and cloth ($R = 0.29$)) are not significantly different from zero at the 5% level. The strong correlation between the pulling forces on glass and perspex can be seen in Fig. 6.

(ii) *Number of adhesive setae*

Some of the specimens viewed with the SEM were covered with debris and their tarsal adhesive setae were too obscured to count. The numbers of adhesive setae on the left-hand tarsi of 14 of the 20 specimens viewed were counted and these are plotted against body weight in Fig. 7. Two types of adhesive setae are present in *C. polita*

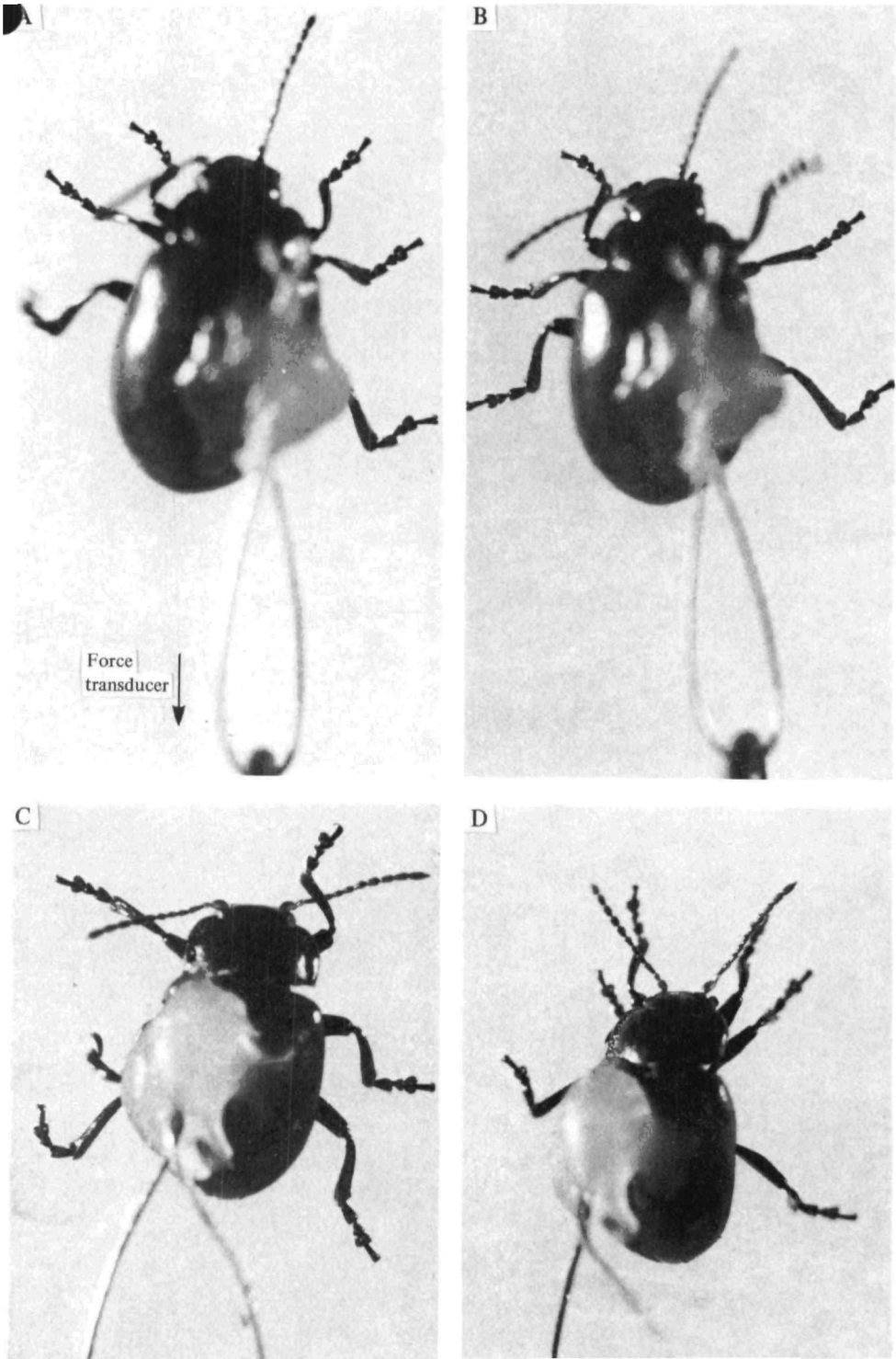


Fig. 1. (A-D) Photographs of *Chrysolina polita* walking vertically up glass, pulling against a force transducer. (A-B) Beetle pulling near or at its maximum pulling force and the tarsi on each side are in roughly the same positions. Note only one tarsus is moving at any one time. (C) An unrestrained beetle walking up glass. (D) Beetle being pulled down, with its tarsi splayed behind it.

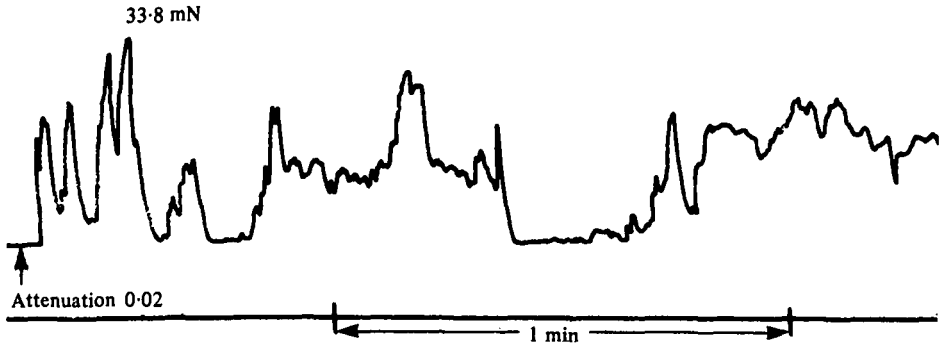


Fig. 2. Oscillograph recording of the pulling force for a male *Chrysolina polita* on glass. The maximum recorded force for this trace is 33.8 mN.

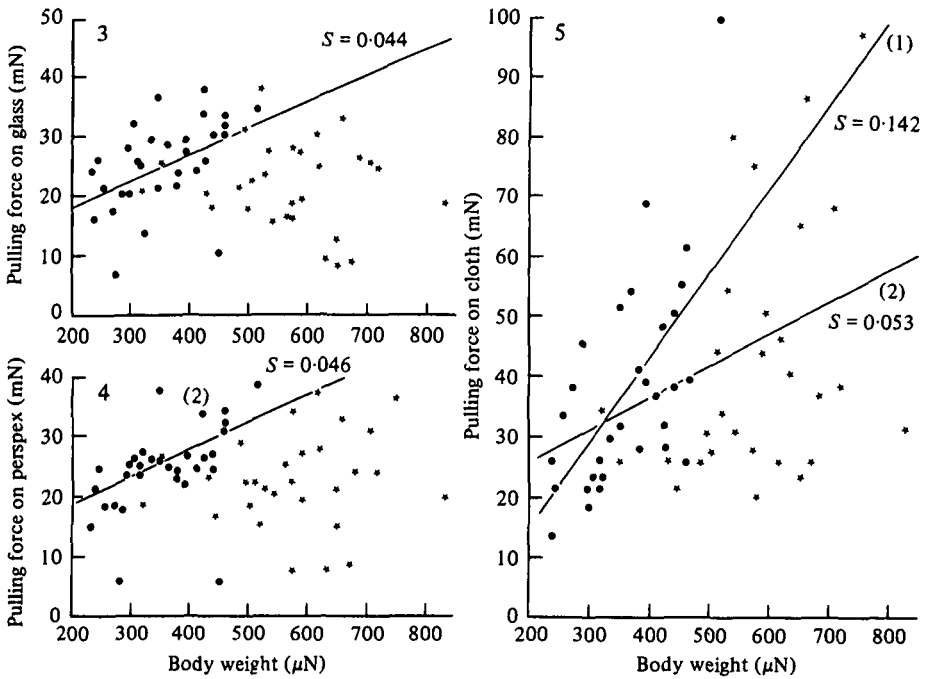


Fig. 3. Pulling force on glass against body weight in *Chrysolina polita*. The regression line for the males (black circles) (regression coefficient = 0.044) is significant at 1% level. Females - stars.

Fig. 4. Pulling force on perspex against body weight in *Chrysolina polita*. The regression line for the males (black circles) (regression coefficient = 0.046) is significant at 1% level. Females - stars.

Fig. 5. Pulling force on cloth against body weight in *Chrysolina polita*. Regression line 1 (males, black circles, regression coefficient = 0.142) is significant at 0.1% level. Regression line 2 for the males and females (regression coefficient = 0.053) is significant at 1% level. Females - stars.

Table 1

(A) General experiment								
Males					Females			
	B. wt.	Glass	Perspex	Cloth	B. wt.	Glass	Perspex	Cloth
<i>M</i>	35.89	25.26	25.19	38.01	57.62	22.28	22.66	42.97
S.E.	1.38	1.32	1.30	3.18	2.04	1.44	1.43	3.92
<i>N</i>	(32)	(31)	(32)	(31)	(30)	(30)	(29)	(29)

(B) Removal of tarsal claws (males and females)							
	B. wt.	Glass		Perspex		Cloth	
		Claws	No claws	Claws	No claws	Claws	No claws
<i>M</i>	39.47	22.40	23.65	24.39	25.79	28.65	4.41
S.E.	3.10	1.61	1.60	1.81	2.57	2.03	0.74
<i>N</i>	(15)	(15)	(15)	(15)	(15)	(15)	(15)

(C) Reduction of electrostatic forces (males and females)					
	Gold				
	B. wt.	glass	Glass	Perspex	Cloth
<i>M</i>	43.56	24.71	19.27	20.87	46.17
S.E.	5.49	1.94	2.06	2.24	7.51
<i>N</i>	(10)	(9)	(10)	(9)	(9)

(D)							
	Cleaning glass (males and females)			Dry atmosphere (males and females)			
	B. Wt.	'Dirty'	'Clean'	B. Wt.	Glass	B. wt. (control)	Glass (control)
<i>M</i>	48.58	17.49	19.39	45.99	25.91	43.53	23.76
S.E.	5.83	1.91	1.51	5.05	1.28	4.53	1.36
<i>N</i>	(11)	(11)	(11)	(12)	(12)	(16)	(16)

All body weights (B. Wt.) $\times 10^{-5}$ N, all remaining figures are pulling forces $\times 10^{-8}$ N.
M = mean, S.E. = standard error, *N* = number of individuals.

first, climbing setae, which are found in both sexes, and secondly, male setae, which are only found in the males and are used to aid pairing. In Fig. 7 the numbers of male setae and climbing setae are shown separately and combined. Regression lines are shown for the numbers of (i) male setae, (ii) male and climbing setae (combined) in males, (iii) climbing setae in females, and (iv) climbing setae in males and females (combined), since their regression coefficients are significantly different from zero at, at least, the 5% level. In all these groups the numbers of adhesive setae increase with body weight. In Fig. 8 the pulling forces on glass are plotted against number of adhesive setae (male and climbing (combined) in males). Regression lines are fitted for females and males and females (combined) since their regression coefficients are significantly different from zero at the 5% level. In females and males and females (combined) the pulling force on glass increases with number of adhesive setae.

(iii) Removal of tarsal claws

Removal of the tarsal claws appeared to have little obvious detrimental effect on the beetles. Tables 1 and 2 show that claw removal has no significant effect on the pulling

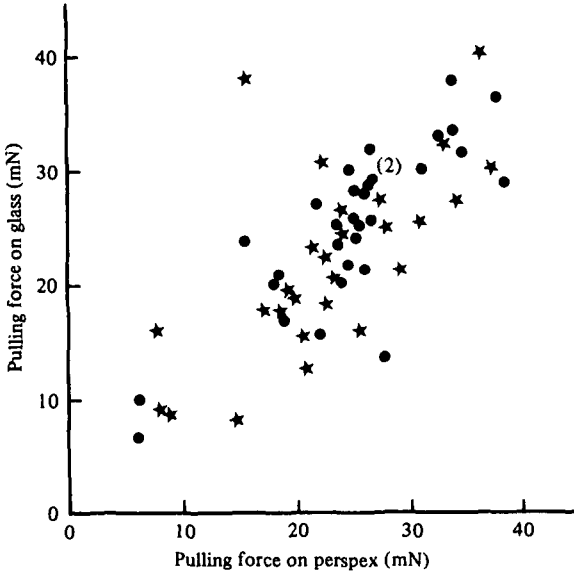


Fig. 6. Pulling force on glass against pulling force on perspex for *Chrysolina polita*. Correlation coefficients (R) for males (black circles) ($R = 0.83$), females (stars) ($R = 0.68$) and males and females ($R = 0.76$) all significant at 0.1 % level.

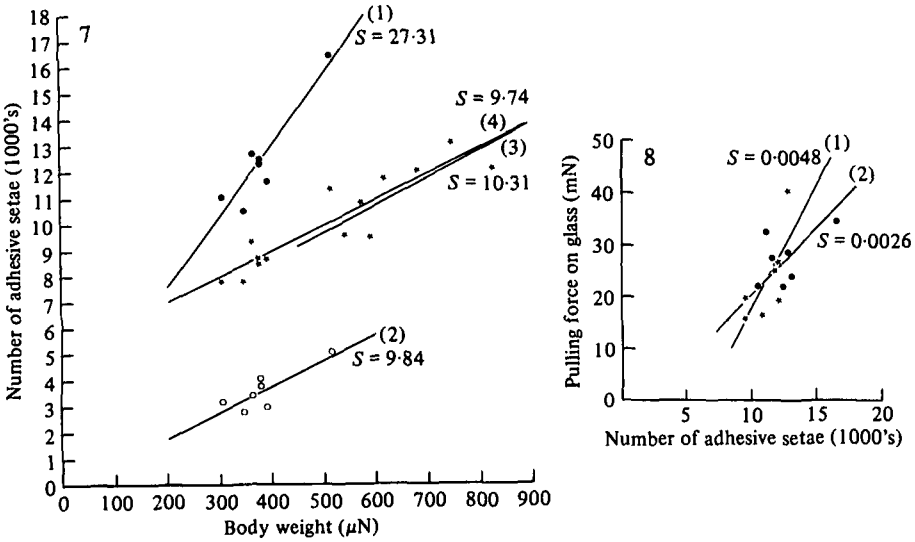


Fig. 7. Number of adhesive setae against body weight in *Chrysolina polita*. Regression line (1) for setae in males (regression coefficient (S) = 27.31) significant at 0.1 % level, regression lines (2) for male setae in males ($S = 9.84$), (3) for climbing setae in females ($S = 10.31$), and (4) for climbing setae in males and females (combined) ($S = 9.74$) are significant at 5 % level. Male setae (open circles), climbing setae (stars), male and climbing setae combined (black circles).

Fig. 8. Pulling force on glass against number of adhesive setae in *Chrysolina polita*. Regression lines 1 for females (regression coefficient (S) = 0.0048) and 2 for males and females (combined) ($S = 0.0026$) are significant at the 5 % level. Females, stars; males, black circles.

Table 2. *Results of the Multiple regression analysis*

- (1) Analysis of the pulling forces of 14 individuals on glass with body weight, sex and number of adhesive setae:

pulling force = $-6.19 + 0.0026$ setae
 (0.0026 ± 0.00091 (S.E.) probability 0.013, significant at 5 % level,
 body weight and sex have no significant effect on pulling force)

Multiple regression coefficients for:

- (2) The effect of claw removal when pulling on:
 glass = -1.253 ± 2.266 (probability 0.585, not significant)
 perspex = -1.393 ± 3.229 (probability 0.670, not significant)
 cloth = 24.240 ± 2.211 (probability 0.000, significant at 0.1 % level)
- (3) The effect of removal of electrostatic forces when pulling on:
 glass = -4.024 ± 2.664 (probability 0.125, not significant)
 perspex = -9.014 ± 2.581 (probability 0.728, not significant)
 cloth = 4.779 ± 6.220 (probability 0.445, not significant)
 'gold' glass = 1.511 ± 2.639 (probability 0.569, not significant)
- (4) The effect of cleaning dirty glass on pulling forces:
 = 1.900 ± 2.623 (probability 0.412, not significant)
- (5) The effect of dry atmosphere on pulling forces on:
 glass = 2.290 ± 1.844 (probability 0.226, not significant)

forces achieved on glass and perspex but results in a significant (at the 0.1 level) reduction in pulling force on cloth.

(iv) *Suction*

There was no obvious reduction in pulling force on glass for any of the ten individuals used when the pressure of the surrounding atmosphere was reduced. In a few cases the pressure reduction caused an increase in the beetle's activity and thus slightly greater pulling forces were recorded than at normal atmospheric pressure. On no occasion did the beetles fall off the glass. An example of an oscillograph trace recorded in this experiment is shown in Fig. 9.

(v) *Reduction of electrostatic forces*

The means and standard errors of the pulling forces on glass finely coated with gold, and glass, perspex and cloth, all with electrostatic forces reduced by use of the Zerostat antistatic gun, are presented in Table 1. Comparison by multiple regression of these results with the pulling forces achieved under normal conditions on the three substrates (Table 2) shows that the reduction of electrostatic forces does not have a significant effect (at the 5 % level) on pulling force.

(vi) *Clean and dirty glass*

The means and standard errors, of the pulling forces, before and after cleaning the sheet of glass, and a multiple regression analysis of these results are shown in Tables 1 and 2, respectively. The multiple regression analysis shows that no significant (at the 5 % level) reduction or increase in pulling force is produced by cleaning the sheet of glass with acetone and absolute alcohol.

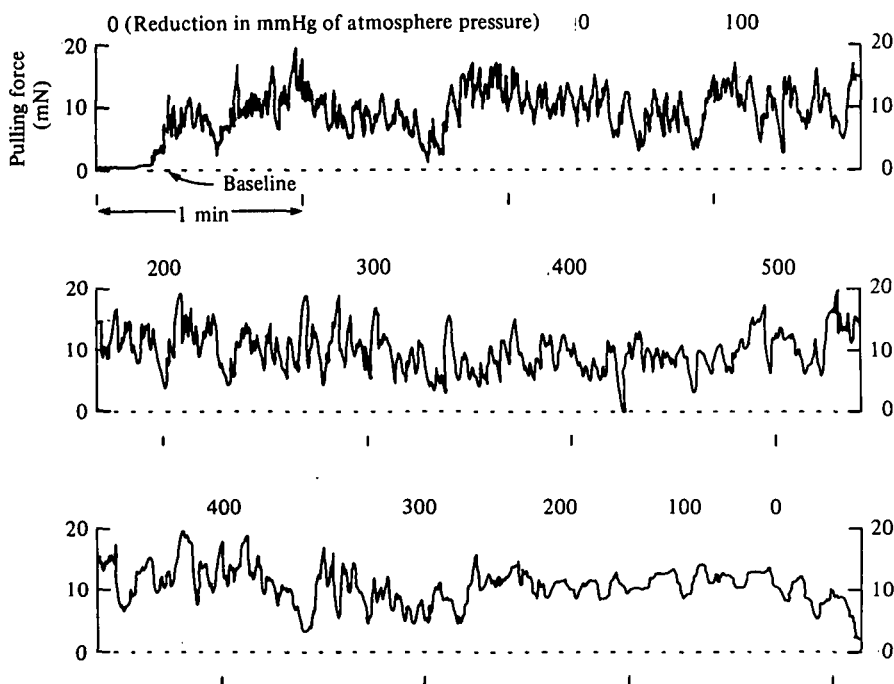


Fig. 9. A continuous oscillograph recording of the pulling force of a female *Chrysolina polita*, weighing 57.7×10^{-8} N, adhering to glass under conditions of reduced atmospheric pressure.

(vii) Dry atmosphere

The means and standard errors of the pulling forces on glass recorded in the dry atmosphere and similar data of normal pulling forces recorded in the same week, are presented in Table 1. Comparison of this data by multiple regression analysis, presented in Table 2, shows that there is no significant effect (at the 5% level) on pulling force produced by using a dry atmosphere.

DISCUSSION

(a) Pulling force and number of adhesive setae

In this discussion the pulling forces of *C. polita* are used as indicators of the likely adhesive forces attainable by adhesive setae in this species. It is important, therefore, that the relationship between these two forces be outlined. Pulling force is equal to the frictional force between the tarsi and the substratum, both forces acting parallel to the plane of the surface. The relationship of pulling force to adhesive force (which acts in a plane at right angles to that of the substratum) may be considered at the simplest level by taking moments about the hind tarsi of a pulling beetle.

$$F \times B = P \times D,$$

where F = adhesive force, P = pulling force, B = distance between front and hind tarsi and D = perpendicular distance of the pulling force from the substratum. As B is usually more or less the same as D , then the pulling force and adhesive force are also similar.

The main forces of detachment acting on an attached leaf beetle are probably drag and the whiplash effect of the leaf in a wind (see later). One might expect body weight to be correlated with adhesive force (and hence pulling force) to counter the increased detachment forces acting on larger and heavier beetles. This appears to be the case for male *C. polita*, where there is a significant increase in pulling force on glass, perspex and cloth with body weight, but not for the females. On dissection of the beetles (when sexing them) it was noted that many of the females, but not all, bore considerable numbers of eggs, and that the size and number of the eggs varied from individual to individual. Although no estimates were made of the contribution the eggs made to the body weights, it is probable that this was considerable. This is reflected in the much larger distribution of body weights in the females than in the males, as evidenced by the ratio of variance to mean of body weights which for the females ($124.4/57.6 = 2.16$) is much larger than for the males ($60.6/35.9 = 1.68$). Thus the results for the females in Figs 3–5 are distorted by the fact that there are two populations present; gravid and non-gravid individuals. Both produce similar pulling forces (since there is no reason that the extra weight of the eggs should increase the pulling force) but their weight distributions are separated.

The pulling force on glass increases significantly with the total number of adhesive setae in females and males and females (combined); (Fig. 8) with more data it is probable that this would prove to be the case for males. The multiple regression analysis (Table 2) confirms the view that pulling force on glass is dependent on the number of setae and that body weight and sex have no significant effect. When the numbers of adhesive setae are plotted against body weight, both males and females show a significant increase in total number of setae with body weight but not when combined (Fig. 7). In addition, the general range of the total numbers of adhesive setae are similar in both sexes, as are the means and ranges of pulling forces on smooth surfaces (Table 1, Figs. 3 and 4). However, the females are considerably heavier than the males (females mean body weight = 57.6×10^{-5} N, range 31.9 – 82.7×10^{-5} N, males 35.9×10^{-5} N, 23.4 – 51.6×10^{-5} N) and one might expect them to require greater pulling forces to counter the effect of increased detachment forces. Climbing setae have long 'semi-rigid' shafts but very flexible plates (the distal parts of the setae used in adhesion), often bearing small projections on the dorsal, non-adhesive surface, whereas male setae have more rigid shafts and larger discoidal plates (Stork, 1980). The male setae are probably specialized for adhering to certain smooth areas of the female's hind body and almost certainly adapt less well to the irregular topography of plant cuticles than the more flexible climbing setae. A more accurate measure of the beetle's possible resistance to detachment forces whilst on plant surfaces may be obtained by observing the relationship of numbers of climbing setae (alone) with body weight (Fig. 7). It is evident that the numbers of climbing setae in females are greater than those in males and also that (in spite of the effect of the extra weight of the eggs in the gravid females) the number of climbing setae increase significantly with body weight in males and females (combined). It is worth noting that during pairing the female carries the male and as a result may have to resist proportionally greater detachment forces.

Ignoring differences in relative size and shape of the setae (Stork, 1980) one can use the regression coefficient in Fig. 7 to estimate that the number of adhesive setae in, for example, a male *Tomarcha tenebricosa* (Fabricus) (Chrysomelidae) weighing abo

3.9 mN is about 106500. Stork (1977) visually estimated the number of adhesive setae for a specimen of this size as about 100000 using the SEM. The fact that these two estimates are so close suggests that the number of adhesive setae is related to body weight not only in *C. polita* but also in other chrysomelids. Similarly, a pulling force on glass of 1.73 N can be extrapolated for the same specimen of *T. tenebricosa* using the regression coefficient from Fig. 3. In fact, this figure is much greater than several estimates, using this species, recorded in some preliminary pulling experiments. It is probable that larger beetles such as this species have problems in manipulating their tarsi so that all the setae can come into contact with the substratum.

(b) Mode(s) of adhesion

Most authors who have previously examined adhesion using adhesive setae have tended to support a single mechanism, virtually ignoring the several other possibilities. (e.g. Gillett & Wigglesworth, 1932). The figures for pulling forces in this present paper, if these are accepted as approximations or indicators of adhesive forces, can be used in conjunction with information on the structure of the adhesive setae (Stork, 1980) for probably the first detailed theoretical and practical approach to most of the previously proposed modes of adhesion.

Hooks

Hooke (1667) and Leeuwenhoek (1690) proposed that the adhesive setae of insects could catch on to the minute irregularities of apparently smooth surfaces. Both glass and perspex when viewed with the SEM are remarkably smooth, even at the magnification of the adhesive setae, and SEM observations of setae in contact with glass (Stork, in prep.) show that neither the setae nor the setules they sometimes bear (on the dorsal non-adhesive surface) hook onto the surface. The results of the multiple regression analysis from the claw amputation experiment (Table 2) show that claw removal does not have a significant effect on adhesion to glass or perspex, and indicate that claws play only a minor role, if any, in adhesion to these surfaces. In contrast, the analysis shows, that the claws play a significant role in holding on to cloth, and that their removal drastically affects the beetles' pulling forces. Some preliminary experiments involving the removal of adhesive setae by cauterization support the view that they are essential for climbing smooth surfaces but are not important in climbing cloth. Since adhesion on smooth and rough surfaces is achieved by different methods, it is not surprising that there is strong correlation between the pulling forces on glass and perspex (as shown in Fig. 6) and poor correlation between the pulling forces on these surfaces and those on cloth.

Suction

Derham (1798) was probably the first to suggest that the large cup-shaped adhesive structures on the front tarsi of the male *Dytiscus* hold onto the female's hind body by the exclusion of air. In other words, air between the seta and the substrate is excluded, thus causing an internal pressure deficit which is maintained by the force of the surrounding atmosphere. This method requires that the edges of the cup-shaped structures be well sealed to prevent the intrusion of atmospheric air. Derham and others suggested that each of the adhesive setae in other animals acted as a minute sucker. If

these animals hold onto smooth surfaces by suction then the reduction of atmospheric pressure should result in a drop in their adhesive force. The oscillograph recordings of pulling forces for *C. polita*, as illustrated in Fig. 1, show no such reduction under conditions of reduced pressure.

Let us consider that the average number of adhesive setae per individual *C. polita* is about 12000, and that $40 \mu\text{m}^2$ is an average value for the adhesive area of each seta. One atmosphere (760 mmHg) of pressure = $1.0133 \times 10^5 \text{ N/m}^2$.

$$F = P \times A \times N,$$

where F = maximum force achievable by suction, P = pressure deficit in N/m^2 , A = area of each seta in m^2 and N = number of adhesive setae.

$$\begin{aligned} F &= (1.0133 \times 10^5) (40 \times 10^{-12}) (1200) \\ &= 48.6 \text{ mN}. \end{aligned}$$

In theory therefore the force achievable by suction could be sufficient to account for the mean pulling force on glass for *C. polita* (approximately 24 mN). In practice this is not the case. If we take the typical example of a female *C. polita* weighing about 0.5 mN and bearing approximately 12000 adhesive setae, its pulling force at a pressure reduced by 600 mm of mercury, was recorded as being 30 mN. Here, the surrounding atmospheric pressure for the beetle was approximately 160 mm of mercury ($1 \text{ mmHg} = 1.3332 \times 10^2 \text{ N/m}^2$). In this case,

$$\begin{aligned} F &= (1.3332 \times 10^2 \times 160) (40 \times 10^{-12}) (12000) \\ &= 8.53 \text{ mN}, \end{aligned}$$

which is considerably less than the 30 mN recorded.

Nachtigall (1974) notes that to produce a reduced pressure a force must be applied to pull the centre of the suction cup away from the surface. The adhesive setae in most beetles are normally 'hinged' proximally and are clearly not designed for this purpose. However, a slight reduction in pressure may result from the setal plate (which is probably composed of 'elastic' cuticle), tending to return to its original shape on being applied to the substratum.

Male dytiscids live in an aquatic environment and use adhesive setae to hold on to the females. Under these conditions, adhesion cannot be accounted for by surface tension, and difficulties in excluding the fluid layer between the setae and the female's elytra will almost certainly severely restrict the close contact required for direct molecular cohesion. In this case, the similarity of the dytiscid male setae to suction cups and the fact that they adhere in an aquatic environment makes suction the most likely mode of adhesion. A muscular mechanism need not necessarily be invoked for the production of the negative pressure. Nachtigall (1974) reports that the male 'strikes' the female with its tarsi. The possible elasticity of these large cuticular structures may cause a tendency to return to their original shape on attachment, and this may produce a negative pressure. The secretion produced at the base of the giant adhesive setae, as noted by Korschelt (1923) and Blunck (1912), probably acts as a sealant around the edges of the setae.

Electrostatic forces

When discussing the possible modes of adhesion in gekkos, Maderson (1964) suggested that electrostatic forces between the adhesive hairs and the substratum must be considered as a possibility. The results of the multiple regression analysis (Table 2) show that the reduction of electrostatic forces by use of an antistatic gun or use of earthed gold-coated glass has no significant effect on the pulling forces of *C. polita*. It has been demonstrated that the Zerostat antistatic gun removes about 70–90% of charge on gramophone records and similar amounts of charge, or less, are probably present on the glass and perspex substrates. Electrostatic forces, therefore play a small role, if any, in the production of the adhesive force by adhesive setae.

Cohesive forces and surface tension

Molecules in a fluid are held together by comparatively long-range attractive forces; the forces of cohesion. Blackwall (1830), Dewitz (1883) and other authors proposed that flies and other insects secrete a sticky fluid and that the cohesive forces of this fluid hold the setae onto smooth surfaces. Since the forces of attraction between solid surfaces and fluid molecules (adhesive forces) are usually greater than the cohesive forces between the molecules of the fluid, then if the two solids are pulled apart any 'tear' is most likely to occur within the fluid. The force resisting this tendency to tear is surface tension and results from the cohesive forces of the fluid. It is caused by the tendency of molecules to have an overall directional force into the body of the fluid (i.e. away from the surface) at a boundary with a gas. Many authors believe that secretions are used to produce the adhesive force by surface tension. However, it has been suggested that almost all surfaces of solids bear minute traces of adsorbed water vapour and other fluids, under normal atmospheric conditions (Bikerman, 1958), and the presence of secretions need not necessarily be invoked. The reduction of pressure within a fluid resulting from the surface tension, when separating a flat circular plate from a flat surface, is given by:

$$P = S \left(\frac{1}{R} - \frac{1}{r} \right) \quad (1)$$

where P = internal pressure, S = surface tension of the fluid, R = radius of the circular plate, r = half the thickness of the fluid layer, F = adhesive force, and A = area of circular plate (Fender, 1954).

Therefore,

$$F = P \times A.$$

When R is large compared to r then in (1) the reciprocal of R can be virtually ignored in comparison with that of r :

$$\text{therefore, } F = P \times A, \simeq \frac{S \times A}{r}. \quad (2)$$

The surface tension of pure water is 73 mN/m and Davies & Rideal (1961) suggest that the thickness of a monomolecular layer of fluid is about 1 nm. If we consider the adhesive plates to be circular then in an average *C. polita* the maximum possible force

holding them on to the surface by surface tension, when separated by a layer of pure water

$$= \frac{(73 \times 10^{-3}) (40 \times 10^{-12} \times 12000)}{0.5 \times 10^{-9}}$$

$$= 70.08 \text{ Newtons.}$$

This figure is 10^3 times larger than the average pulling force for *C. polita* (approx. 24 mN), but several important assumptions have been made in arriving at this figure. It has been assumed that all the setae are in contact, which from observation is rarely the case. In addition, the fluid adsorbed on a surface is probably 'polluted' by dirt and dust particles and its surface tension will be lower than that for pure water. Finally, the setal plates and the substrates, although fairly flat at the microscopic level, are in fact very coarse at the molecular level. Separations of 1 nm probably only occur at a limited number of points for each seta. Thus, if the fluid layer is monomolecular the area available for surface tension will be much less than that estimated above. Equation (2) shows that the force achieved by surface tension is inversely related to the thickness of the fluid layer. This somewhat counteracts the fact that a thicker layer of fluid would increase the area of contact. Clearly, the figure of 70.08 N is a large overestimate and cannot be taken too seriously.

The results from the multiple regression analysis show that cleaning the sheet of glass used in the pulling experiments with acetone, and attempting to remove as much water vapour as possible by measuring pulling forces in a dry atmosphere, does not have a significant effect on the pulling forces of *C. polita*. However, it is extremely difficult to remove absorbed water vapour from surfaces such as glass and perspex and these experiments in no way rule out the possibility of surface tension producing the adhesive force. There may even be sufficient fluid present on the setal plates for surface tension effects to occur.

A direct measure of the cohesive force of a fluid is its ultimate tensile strength. The tensile strength of water is an important factor in the transport of water in plants and negative pressures of up to 80 atm have been recorded by Scholander *et al.* (1965) in twigs from creosote bushes. Theoretical values for tensile strengths of fluids are much greater than those actually recorded because of impurities in the fluid. If we take the example of 80 atm as being the tensile strength of a fluid on a smooth surface then the pulling force achievable can be estimated using equation (2):

$$F = P \times A = (80 \times 1.0133 \times 10^5) (40 \times 10^{-12} \times 12000)$$

$$= 3.89 \text{ N.}$$

However, this figure is less than the estimate of 70.08 N for the force achievable by surface tension. This means that the fluid layer would 'crack' before the latter figure was reached. Here the ultimate tensile strength of the fluid is the limiting factor. For surface tension to be the limiting factor the minimum thickness of the fluid would have to be greater than 9.0 nm in this case. In more general terms, the resisting force that must be overcome is the ultimate tensile strength of the fluid for thin layers, and surface tension for thicker layers of fluid.

Seizure

Gillett & Wigglesworth (1932) suggested that adult *Rhodnius prolixus* Stal (Hemiptera: Reduviidae) hold on to smooth surfaces by means of 'seizure' between the adhesive setae on their front tibiae and the substratum. They proposed that the dorsal surface of each seta made contact at an angle with the substratum and that a droplet of fluid was secreted in this angle. On pulling the tibiae, and thus the setae, towards the body, thinning of the fluid at the tip of each seta results in these surfaces coming into very close contact. Molecular adhesion (see below) at these points causes seizure to occur. However, SEM observations of setae adhering to glass in *C. polita* (in prep.) indicate that it is the ventral surface of the seta that comes into contact with the substratum, and seizure cannot occur. It is improbable that seizure accounts for adhesion in any animal using adhesive setae. Indeed, the dorsal surface of the adhesive setae in *R. prolixus* and in some flies have been observed to bear small projections similar to the setules in some beetles (Stork, unpublished).

Molecular adhesion

Ruibal & Ernst (1965) noted that *Gekko gekko* may bear 10^8 – 10^9 spatulae on the hairs on their tarsi. They suggested, as did Edwards & Tarkanian (1970) for *R. prolixus*, that direct 'frictional' forces between the spatulae and the substratum provided the adhesive force. Various forms of molecular interactions at the points of contact cause the molecules of two surfaces to be loosely or strongly bonded together. One example, which is bound to exist even if no others are present, is a group of relatively weak long range forces called van der Waal's forces. Molecules are weakly attracted to each other over distances of a few Ångströms by the van der Waal's forces, but at smaller distances a repulsive force occurs. If sufficiently large numbers of molecules are effectively in contact (i.e. separated by a few Ångströms) the attractive van der Waals' forces may be considerable and molecular adhesion may occur. Without knowing the number of points of contact and the separation of the setae from the substratum a mathematical analysis of the feasibility of these forces as the mode of adhesion is not possible. However, the fact that there are so many individual adhesive units in beetles and in gekkos, and that the separation of the setae from the substratum has been demonstrated to be less than 100 nm (Stork, unpubl.), is perhaps strong evidence to support molecular adhesion as the adhesive force.

Histological investigations of the tarsi of *C. polita* by the present author have failed to show any obvious secretory cells. Although several genera of chrysomelids are noted for their copious production of a tarsal secretion there appears to be little evidence of similar secretions in most beetles. Recently, Hasenfuss (1978) and Bauchhenb & Renner (1977) have supported the views of many 19th century authors that flies produce an adhesive secretion. Clearly, further electron microscope studies are required to determine whether secretions are produced.

In conclusion, the most probable mode of adhesion on smooth surfaces in *Chrysolina polita*, and perhaps in most animals using adhesive setae, is direct molecular adhesion of the setae with the substratum. It is probable that the cohesive forces of a thin fluid layer would increase this adhesion. If a thicker layer of fluid is present then adhesion by direct molecular adhesion and surface tension will be considerably

reduced. Whether most beetles produce secretions to aid adhesion on smooth surfaces is uncertain but perhaps this may occur in some genera and other groups such as flies. Finally, the unique structure of some male adhesive organs in aquatic caraboids, and the fact that these are used in an aquatic environment, makes adhesion by suction a distinct possibility in this group.

(c) *Detachment forces*

The forces of detachment acting on a leaf beetle must be considerable if the pulling force (and therefore probably the adhesion) for an average specimen of *C. polita* on a smooth surface is about fifty times its body weight. The effect of drag and whiplash as forces of detachment perhaps can be considered theoretically in isolation. Assuming a drag coefficient of 1:

$$D = 0.5 \rho V^2 AC_D$$

(Alexander, 1971, following his reasoning on p. 50),

where V = velocity of wind, C_D = drag coefficient, ρ = density of air, D = drag, A = cross-sectional area of the beetle on which the wind is acting.

Using the above equation for an average individual *C. polita* (body weight about 45×10^{-5} N, cross-sectional area approximately 16×10^{-6} m²), the estimated wind speeds required to remove the beetle solely by drag, equivalent to the pulling forces of 24 mN on glass and 40 mN on cloth, are about 48 m/s. (107 m.p.h.) and 62 m/s. (137 m.p.h.) respectively.

Similarly, if we consider that leaves and branches in a gusty wind oscillate in a simple harmonic motion:

$$\text{maximum acceleration} = 4\pi^2 \times a \times f^2,$$

where a = amplitude, f = frequency,

and maximum force on a beetle of mass $M = 4\pi^2 \times a \times f^2 \times M$.

On a branch vibrating at 2 Hz and 0.1 m, for instance (this probably represents the effect of a strong gusty wind), then the maximum force

$$= 4\pi^2 \times 0.1 \times 4 \times M \simeq 16M.$$

From these calculations it is clear that drag and the whiplash of leaves and branches in a gusty wind may be important as detachment forces acting on a beetle.

I would like to thank Dr R. F. Ker and Prof. R. McN. Alexander (University of Leeds) for their helpful discussion and criticism of an earlier draft of this manuscript. I am also grateful to Dr M. E. G. Evans and Dr I. Grant (University of Manchester) for their advice, to Mr L. Lockey for photographic assistance, and to Prof. E. R. Trueman for the use of departmental research facilities. This research was carried out under an S.R.C. award.

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