cotized with 0.1 percent nicotine, after feeding to prevent regurgitation or defecation of ingested particles, preserved with Formalin, and cleared with sodium hypochlorite. All other organisms were preserved with mercuric chloride. Crustaceans were enumerated in samples obtained by towing 75-μm net four times in the epilimnion (0 to 5 m). At least 400 animals were counted per tow. At least 50 rotifers and 50 protozoans were enumerated in each of three integrated whole-water samples drawn with a Nalgene tube (inner diameter, 2.5 cm). Bead uptake was determined for 80 crustaceans, 80 rotifers, 50 ciliates, and 196 *Dinobryon* cells. Abundant genera were *Mesocyclops*, *Daphnia*, and *Bosmina* (Crustacea), *Conochilus* and *Keratella* (Rotifera), and *Halteria* (Ciliata). The *Dinobryon* population was largely *D. bavaricum* with some *D. sociale*.


11. W. D. Taylor *et al.*, *U.S. Environ. Prot. Agency, Natl. Eutroph. Surv. Work. Pap. No. 710* (1979).
12. S. C. Hern *et al.*, *U.S. Environ. Prot. Agency, Natl. Eutroph. Surv. Work. Pap. No. 707* (1978).



13. We thank G. Nurnberg for translation from German, M. Neuwirth for electron microscopy, R. Lamarche for photographic assistance, the Université de Montréal for use of their field station, and R. H. Peters and W. C. Leggett for critical reviews. Supported by Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant to J. Kalff, an NSERC postgraduate scholarship to D. Bird, and by the generosity of Mrs. J. R. Routledge.

7 June 1985; accepted 30 September 1985


# Diet-Induced Head Allometry Among Foliage-Chewing Insects and Its Importance for Graminivores


E. A. BERNAYS



**Individuals of the grass-feeding caterpillar of *Pseudaletia unipuncta*, reared from hatching on hard grass, had head masses twice as great as those of caterpillars fed soft artificial diet, even though the larvae reached the same body mass. Larvae reared on soft wheat seedlings had intermediate head masses. Thus muscular effort increases muscular development in an insect, which in turn has a dramatic morphogenetic effect on head size. Size differences in the head capsules, with the correlated differences in mandibular power, have a direct effect on the ability of the insects to ingest hard foods rapidly: larger heads are adaptive for dealing with hard grasses.**


JAW AND TOOTH ADAPTATIONS ASSOCIated with grass feeding occur in a number of animal groups. Most studies of grass-feeding specialists among the insects have focused on grasshoppers. Mandibles of grass-specializing grasshoppers show chisel-like incisors and flattened, grooved molar cusps, analogous to the adaptations of teeth in grazing mammals (*1*). Among caterpillars, fewer studies have been undertaken, although taxonomic studies in the genus *Spodoptera* (*2*) show that the grass specialists have mandibles with chisel-like edges while other species have pointed incisor cusps. In the study reported here, the allometric consequences of food choice were investigated.

A total of 82 grasshoppers and 76 caterpillars collected in North America and Australia were examined (*3*). Gut contents were removed and the body, head, and mandibles of each were dried and weighed. In both groups, grass specialists consistently had a greater head mass than other foliage feeders of similar body sizes (Fig. 1). Among grasshoppers the proportionality coefficients were 0.22 ± 0.04 (mean ± standard error) and 0.12 ± 0.04 for grass specialists and non–grass specialists, respectively, while among caterpillars the figures were 0.15 ± 0.04 and 0.04 ± 0.03, indicating a much greater difference in the latter group.

Relative head mass also varies intraspecifically in relation to diet. The effect of diet on head development in the grass-feeding caterpillars of the noctuid *Pseudaletia unipuncta* was studied, with some additional experiments on two acridids, *Locusta migratoria* and *Chorthippus curtipennis*. The diets were (i) artificial (extremely soft), (ii) *Poa* and *Triticum* seedlings (relatively soft $C_3$ grasses), and (iii) mature *Zea*, *Bambusa*, and *Cynodon* (hard $C_4$ grasses) (*4*).

Table 1. Effect of diet on relative head size in larvae of *P. unipuncta*. Values are means ± standard errors.

| Rearing diet | Number | Head dry mass (percentage of total body dry mass) |
|---|---|---|
| | *Experiment 1* | |
| *Triticum* | 20 | 11.9 ± 0.5 |
| *Zea* | 20 | 14.7 ± 1.4 |
| | *Experiment 2* | |
| Artificial diet | 20 | 8.1 ± 0.3 |
| *Triticum* | 23 | 12.3 ± 0.4 |
| *Cynodon* | 22 | 15.6 ± 1.6 |

Caterpillars of *P. unipuncta* were raised from hatching on one of the three diets. Relative head masses were measured in the final larval instar (*5*). Differences in head mass were extreme (Fig. 2); each group was significantly different from the others ($P < 0.005$, $F$ test on coincidence of lines). Caterpillars reared on *Triticum* seedlings or *Zea* showed a similar pattern (Table 1), while heads from the three-diet experiment showed significant differences in morphometrics and in the surface area of attachment of mandibular adductor muscles (Table 2) (*6*). Diet had induced major changes in the caterpillars during growth.

*Locusta migratoria* larvae were reared on *Poa* or *Bambusa* (*7*), while *C. curtipennis* larvae were reared on *Triticum* or *Zea* (*8*). In both cases insects reared on the harder grass had relatively larger heads, although they also had lower masses than those reared on softer grasses and the exponents were significantly greater in the regression analysis. Comparison in terms of head mass as a percentage of total body mass gave values of 22 ± 1 and 17 ± 2 percent (means ± standard errors) for *C. curtipennis* reared on *Zea* and *Triticum*, respectively, and 25 ± 2 and 21 ± 2 percent for *L. migratoria* reared on *Bambusa* and *Poa*, respectively.

That grasses are difficult to chew is generally accepted, the most important feature being the parallel arrays of sclerenchyma fibers (*9*). In addition, high silica levels are presumed to cause wear of cutting and

Table 2. Head morphometrics of fifth-instar larvae of *P. unipuncta* after rearing on three different diets. Insects were of similar total body mass. Values (means ± standard errors) in each column are significantly different from one another ($P < 0.005$, $t$-test).

| Mandible base to top of cranium (mm) | Maximum head width (mm) | Surface area of mandibular adductor muscle attachments to cranium ($mm^2$) |
|---|---|---|
| | *Artificial diet* | |
| 2.01 ± 0.01 ($n$ = 10) | 1.98 ± 0.02 ($n$ = 10) | 2.8 ± 0.3 ($n$ = 5) |
| | *Triticum* | |
| 2.16 ± 0.02 ($n$ = 10) | 2.15 ± 0.01 ($n$ = 10) | 3.6 ± 0.3 ($n$ = 5) |
| | *Cynodon* | |
| 2.45 ± 0.02 ($n$ = 10) | 2.43 ± 0.02 ($n$ = 10) | 4.6 ± 0.6 ($n$ = 5) |


Division of Biological Control, University of California, Berkeley 94720.

Table 3. Feeding rates of mid-fifth-instar caterpillars of *P. unipuncta* from different diets when given a test meal of the hard blades of *Cynodon*.

| Rearing diet | Intake rate (mg/min) | Initial insect mass (mg) |
|---|---|---|
| | *Experiment 1* | |
| *Triticum* | 0.86 ± 0.1 | 409 ± 8 |
| *Zea* | 1.19 ± 0.05* | 403 ± 17 |
| | *Experiment 2* | |
| *Triticum* | 1.12 ± 0.08 | 432 ± 19 |
| *Zea* | 1.32 ± 0.12* | 398 ± 20 |

*Significantly different from corresponding value for *Triticum* ($P < 0.01$, Wilcoxon's test).

grinding surfaces. Grass-chewing insects must make incisions and must chop or grind to aid in the extraction of nutrients (*10*).

Head mass in turn was found to be closely related to mandible mass, irrespective of taxon or feeding habit, as illustrated by the overall allometric equation $M_m = 0.1(\pm 0.008)M_h^{1.03}$ ($n = 179$) ($r^2 = 0.88$), where $M_m$ is mandibular mass and $M_h$ is head mass (both in milligrams). This may be expected, since the mandibular adductor muscles occupy most of the head space in chewing insects (*11*), and the greater head development results from larger mandibles and mandibular adductors.

The harder, tougher grass blades are also likely to be lowest in nutrient content (*10*), so the insects feeding on them not only have the physical problem, but also need to process it more extensively than softer grass. Intake rates should be optimized since feeding activity seriously increases risk (*12*). To ascertain the adaptive value of larger heads, feeding rates were measured. *Pseudaletia unipuncta* larvae were reared on *Triticum* or *Zea*, and in the mid-fifth instar were deprived of food for 18 hours before receiving a test meal of *Cynodon* (*13*). The insects that had been reared on the hard grass had significantly higher rates of intake (Table 3).

In insects chewing grass, head mass is approximately doubled and averages an additional 8 to 10 percent of total body mass both across and within species. If the food is also relatively low in protein, as is often the case, it is difficult to process adequate quantities. At some point, increasing hardness and low nutrients make feeding on such materials subject to a law of diminishing returns, as suggested by the results with the grasshoppers feeding on *Zea* and *Bambusa*. Thus increased throughput of food to make up for poor quality (*14*) may result in stunted adults. Even for grass specialists, certain grasses would seem unsuitable.

The relaxation of maximum head development in *P. unipuncta* on artificial diet was pronounced. If the insects are forced to move from a soft diet to a hard one during larval life, problems may be encountered at least temporarily. Additional genetic changes are being investigated, since some insects reared for many generations on artificial diet have been reported to be poor at feeding on natural foliage (*15*).

From the taxonomic point of view, the morphometric differences due to hardness of diet may need to be examined further. Among locusts, some morphometric phase differences have been attributed to different activity levels (*16*), and gregarious forms that eat more also have relatively large heads (*17*).

The environmental variance components of morphology in invertebrates have received relatively little attention. For example, analogous effects of diet on growth of masticatory muscles in mammals have been known for years (*18*). An increase in muscle size with work is not surprising, but it has not, to our knowledge, been studied in insects. Even during an instar, muscle growth inside the head capsule, which is unexpandable, must be continuing at the expense of air sac space; there must also be an effect on the epidermis, resulting in increased cuticle production. Additional cuticle may be laid down in the same instar to increase thickness, but there must be a large increase in the cuticle of the developing pharate instar to allow for the formation of a considerably larger head. It is also possible that additional instars occur. Further research is needed on the manner in which muscular activity influences the size of cuticular structures, particularly since it may be important for all cuticular structures and not just for the head.

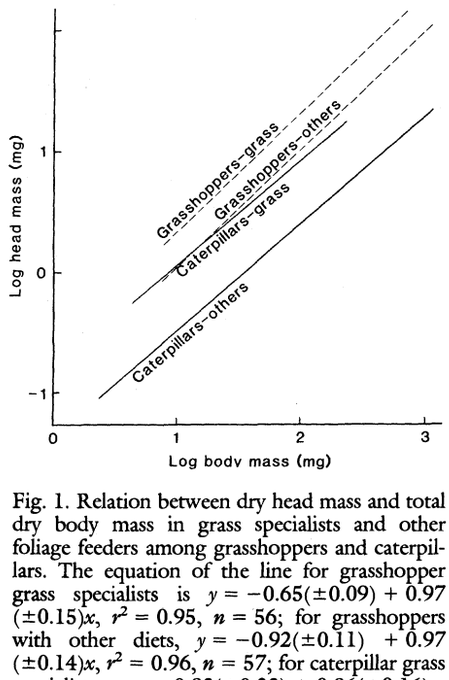


Fig. 1. Relation between dry head mass and total dry body mass in grass specialists and other foliage feeders among grasshoppers and caterpillars. The equation of the line for grasshopper grass specialists is $y = -0.65(\pm 0.09) + 0.97(\pm 0.15)x$, $r^2 = 0.95$, $n = 56$; for grasshoppers with other diets, $y = -0.92(\pm 0.11) + 0.97(\pm 0.14)x$, $r^2 = 0.96$, $n = 57$; for caterpillar grass specialists, $y = -0.83(\pm 0.23) + 0.86(\pm 0.16)x$, $r^2 = 0.64$, $n = 17$; and for caterpillars feeding on other herbaceous plants, $y = -1.37(\pm 0.09) + 0.84(\pm 0.06)x$, $r^2 = 0.76$, $n = 56$.

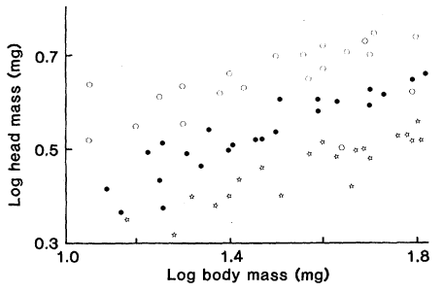


Fig. 2. Relation between dry head mass and total dry body mass in fifth-instar caterpillars of *P. unipuncta* reared from hatching on three different diets. The equation of the line for *Triticum* (●) is $y = -0.001(\pm 0.0005) + 0.365(\pm 0.035)x$, $r^2 = 0.82$; for *Cynodon* (○), $y = 0.058(\pm 0.009) + 0.389(\pm 0.065)x$, $r^2 = 0.62$; and for artificial diet (★), $y = -0.019(\pm 0.001) + 0.306(\pm 0.344)x$, $r^2 = 0.8$.

## REFERENCES AND NOTES


1. F. B. Isely, *Ann. Entomol. Soc. Am.* 37, 47 (1944); L. H. Williams, *Trans. R. Entomol. Soc. London* 105, 423 (1954); S. K. Gangwere, *Trans. Am. Entomol. Soc.* 87, 67 (1961); R. F. Chapman, *Proc. Zool. Soc. London* 142, 107 (1964); P. Hummelon and Y. Gillon, *Ann. Univ. Abidjan* 1, 199 (1968); J. Patterson, *Am. Midl. Nat.* 3, 296 (1984).
2. E. S. Brown and C. F. Dewhurst, *Bull. Entomol. Res.* 65, 221 (1975).
3. The species examined were as follows. Grasshoppers specializing on grasses: *Oxya gavisa* ($n = 3$), *Chorthippus curtipennis* (11), *Laxabilla smaragdina* (4), *Locusta migratoria* (12), *Acrida conica* (2), *Heteropternis obscurella* (2), *Caledia captiva* (3), and *Bermius brachycerus* (1). Grasshoppers with mixed diets or specializing on nongrasses: *Melanoplus devastator* (4), *Melanoplus sanguinipes* (7), *Cibolacris parviceps* (4), *Bootettix punctatus* (2), *Arphia conspersa* (6), *Schistocerca americana* (11), *Atractomorpha similis* (4), and *Valanga irregularis* (6). Caterpillars specializing on grasses: *Pseudaletia unipuncta* (8), *Hemileuca oliviae* (4), *Paratrytone melane* (6), *Coenonymph california* (2), and *Spodoptera exempta* (3). Caterpillars feeding largely on nongrasses: *Manduca sexta* (5), *Phthorimaea operculella* (2), *Spodoptera exigua* (13), *Uresiphita reversalis* (3), *Trichoplusia ni* (8), *Malacosoma californicum* (5), *Pieris rapae* (3), *Danaus plexippus* (2), *Coleus eurytheme* (2), *Estigmene acrea* (8), and *Heliothis zea* (3).
4. Grass hardness was measured with a simple penetrometer on an arbitrary scale with seedling *Triticum* given the value of 1; *Poa annua*, 1; *Zea mays*, 6; *Bambusa* sp., 15; and *Cynodon dactylon*, 4.
5. Pupae from a culture of *Pseudaletia unipuncta* were used: newly hatched larvae were fed artificial diet, lush growing wheat seedlings (*Triticum* 7 to 14 days old), or blades of *C. dactylon* (with cut ends in vials of water), all regularly replenished. Twenty to 24 fifth-instar larvae on each diet were examined.
6. The greatest width and the distance from mandible base to cranium top were monitored on ten heads of fifth-instar larvae on each diet. Other heads were dissected and mandibular adductor muscle attachments drawn.
7. *Locusta migratoria* (University of Queensland, June 1985) third-instar larvae were kept at 30°C, fed daily on *Bambusa* sp. or *P. annua*, and killed just after the molt to the fifth instar.
8. *Chorthippus curtipennis* (University of California, Berkeley, May 1985) second- to third-instar larvae were kept at 30°C, fed mature *Zea* or *Triticum*, and killed within 1 day of final molt.
9. J. F. V. Vincent, *J. Mater. Sci.* 17, 856 (1982).

10. E. A. Bernays and R. Barbehenn, in *Nutritional Ecology of Arthropods*, J. G. Rodriguez and F. Slansky, Eds. (Wiley, New York, in press).
11. R. E. Snodgrass, *Principles of Insect Morphology* (McGraw-Hill, New York, 1935); A. Strenger, *Zool. Jahrb. Abt. Anat. Ontog. Tiere* 75, 1 (1942).
12. J. C. Schultz, in *Variable Plants and Herbivores in Natural and Managed Systems*, R. F. Denno and M. S. McClure, Eds. (Academic Press, New York, 1983).
13. Each individual was weighed and offered fresh *Cynodon*. After feeding began, observations were continued for 15 minutes. Larvae were immediately reweighed and the rate of food intake (in milligrams per minute) was calculated. No feces were produced in the time.
14. N. Moran and W. D. Hamilton, *J. Theor. Biol.* 86, 247 (1980).
15. Y. S. Rathore and W. D. Guthrie, *J. Econ. Entomol.* 66, 1195 (1973).
16. B. P. Uvarov and J. G. Thomas, *Proc. R. Entomol. Soc. London A* 17, 113 (1942).
17. B. P. Uvarov, *Grasshoppers and Locusts* (Anti-Locust Research Centre, London, 1966), vol. 1.
18. W. J. Moore, *J. Zool.* 146, 123 (1965).
19. I thank R. F. Chapman, R. Wrubel, H. Gordon, Y. Tanada, J. Capinera, and staff at the Department of Entomology, University of Queensland.

26 August 1985; accepted 15 November 1985

# Presence of Nonoxidative Ethanol Metabolism in Human Organs Commonly Damaged by Ethanol Abuse

ELIZABETH A. LAPOSATA AND LOUIS G. LANGE

**Acetaldehyde, the end product of oxidative ethanol metabolism, contributes to alcohol-induced disease in the liver, but cannot account for damage in organs such as the pancreas, heart, or brain, where oxidative metabolism is minimal or absent; nor can it account for the varied patterns of organ damage found in chronic alcoholics. Thus other biochemical mediators may be important in the pathogenesis of alcohol-induced organ damage. Many human organs were found to metabolize ethanol through a recently described nonoxidative pathway to form fatty acid ethyl esters. Organs lacking oxidative alcohol metabolism yet frequently damaged by ethanol abuse have high fatty acid ethyl ester synthetic activities and show substantial transient accumulations of fatty acid ethyl esters. Thus nonoxidative ethanol metabolism in addition to the oxidative pathway may be important in the pathophysiology of ethanol-induced disease in humans.**

THERE ARE OVER 10 MILLION ALCOholics in the United States, costing the economy $60 billion annually (*1*), but no generally accepted mechanism has been proposed that accounts for the propensity of certain individuals to drink to excess or to develop alcohol-related damage to organs. Recent studies demonstrated the existence of a heritable predisposition to abuse alcohol (*2, 3*). Because altered rates of acetaldehyde synthesis or degradation could explain this genetic component to alcohol dependency and organ damage, genetic analyses of the oxidative metabolism of ethanol to acetaldehyde (*4–6*) have been conducted.

It remains unclear why organs other than the liver develop alcohol-induced damage, especially the heart, pancreas, and brain, which lack or show minimal oxidative metabolism of ethanol and therefore are free of substantial acetaldehyde production (*7–9*). Moreover, selectivity of organ damage, such as the occurrence of alcohol-induced cardiomyopathy in the absence of liver or pancreatic disease (*10*), is difficult to understand because the extrahepatic organs damaged by ethanol are perfused with blood containing similar concentrations of acetaldehyde derived from the liver (*11*). Selective damage to organs that lack oxidative alcohol metabolism, therefore, suggests that mechanisms of alcohol-induced injury exist that are intrinsic to extrahepatic organs themselves. This is true especially since a genetic component for such organ injury has been observed in, for example, the brain (*2, 12, 13*). We report that ethanol is metabolized to fatty acid ethyl esters in many human organs. This finding suggests a role for nonoxidative ethanol metabolism in the development of damage to these organs.

Cardiovascular Division at Jewish Hospital, Washington University School of Medicine, St. Louis, MO 63110.

Table 1. Fatty acid ethyl ester synthesis by human organs. Tissue was obtained at autopsy from control subjects, homogenized (10 percent, weight to volume) in 50 m*M* tris-HCl (*p*H 8), and incubated with [$^{14}$C]oleate (20,000 dpm/nmol; final concentration, 0.4 m*M*) and ethanol (final concentration, 200 m*M*) (*16*) for 60 minutes at 37°C. After addition of ethyl [$^{3}$H]oleate as a yield marker and ethyl oleate as a carrier, lipids were extracted with acetone and ethyl [$^{14}$C]oleate, the end product, was quantitated after thin-layer chromatography by scintillation spectrometry. Values are means ± standard errors.

| Organ | *n* | Ethyl oleate produced (nmol g$^{-1}$ hour$^{-1}$ |
|---|---|---|
| Pancreas | 5 | 468 ± 169 |
| Liver | 5 | 137 ± 28 |
| Adipose | 3 | 24 ± 9 |
| Heart | 6 | 11 ± 1 |
| Brain (cerebral cortex) | 4 | 11 ± 2 |
| Skeletal muscle | 3 | 7 ± 2 |
| Aorta | 3 | 7 ± 2 |
| Buffer | 5 | <0.1 |

For fatty acid ethyl ester isolation and gas chromatographic quantitation (*14*) in human organs, samples of heart (left ventricle), adipose tissue (abdominal panniculus), liver, pancreas, thoracic aorta, psoas muscle, cerebral cortex, cerebellum, and testes were obtained postmortem, with the addition of vitreous humor, kidney, and thyroid in some cases. A total of 175 organs were sampled from 20 randomly selected subjects.

The distribution of fatty acid ethyl esters reflected the type of ethanol exposure (Fig. 1). In acutely intoxicated subjects ($n$ = 6), the concentrations of fatty acid ethyl esters were significantly higher than in controls in pancreas, liver, heart, and adipose tissue. Chronic alcoholics ($n$ = 4) with undetectable blood alcohol concentrations had high fatty acid ethyl ester concentrations only in adipose tissue. Fatty acid ethyl esters were also detected in lower concentrations in some control subjects ($n$ = 10), a finding probably due to social drinking within several days of death. Although high concentrations of fatty acid ethyl esters were present in adipose tissue in acutely and chronically intoxicated subjects, differences in the adipose content of the parenchymal organs, as reflected by fat vacuoles, were not responsible for the differences in the concentration of these esters between groups.

Of the parenchymal organs in acutely intoxicated subjects, fatty acid ethyl esters were most abundant in the pancreas, liver, heart, and brain (both cerebellum and cerebral cortex) (175, 121, 37, and 25 nmol/g, respectively), all target organs of ethanol-induced disease (Fig. 1). Lesser concentrations were found in the aorta, skeletal muscle, and testes (18, 13, and 15 nmol/g); the esters were absent in the thyroid, vitreous, and kidney. A typical gas chromatogram disclosed ethyl esters of palmitate, palmitoleate, stearate, oleate, linoleate, and arachidonate (Fig. 2). Moreover, in the acutely intoxicated and control groups, increasing blood alcohol concentration was linearly related to increasing fatty acid ethyl ester