15. S. Nee, R. M. May, P. H. Harvey, *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **344**, 305 (1994).
16. J. Terborgh, R. B. Foster, P. Nuñez, *Ecology* **77**, 561 (1996).
17. H. Kreft, W. Jetz, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 5925 (2007).
18. E. G. Leigh Jr., *J. Theor. Biol.* **90**, 213 (1981).
19. R. E. Ricklefs, *Ecology* **87**, 1424 (2006).
20. D. L. Rabosky, *Ecol. Lett.* **12**, 735 (2009).
21. R. E. Ricklefs, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 1265 (2010).
22. K. J. Gaston, *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **353**, 219 (1998).
23. T. J. Givnish, *J. Ecol.* **87**, 193 (1999).
24. J. H. Connell, *Science* **199**, 1302 (1978).
25. D. H. Janzen, *Am. Nat.* **104**, 501 (1970).
26. L. S. Comita, H. C. Muller-Landau, S. Aguilar, S. P. Hubbell, *Science* **329**, 330 (2010).
27. S. A. Mangan et al., *Nature* **466**, 752 (2010).
28. X. Liu et al., *Ecol. Lett.* 10.1111/j.1461-0248.2011.01715.x (2011).
29. S. Nee, A. O. Mooers, P. H. Harvey, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8322 (1992).
30. N. Rønsted et al., *Proc. Biol. Sci.* **272**, 2593 (2005).
31. R. E. Ricklefs, *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **365**, 1139 (2010).
32. T. J. Givnish, *Taxon* **59**, 1326 (2010).
33. R. E. Ricklefs, S. S. Renner, *Evolution* **48**, 1619 (1994).

Heavy Livestock Grazing Promotes Locust Outbreaks by Lowering Plant Nitrogen Content

Arianne J. Cease,[1]* James J. Elser,[1]* Colleen F. Ford,[1] Shuguang Hao,[2] Le Kang,[2]* Jon F. Harrison[1]*

Current paradigms generally assume that increased plant nitrogen (N) should enhance herbivore performance by relieving protein limitation, increasing herbivorous insect populations. We show, in contrast to this scenario, that host plant N enrichment and high-protein artificial diets decreased the size and viability of *Oedaleus asiaticus*, a dominant locust of north Asian grasslands. This locust preferred plants with low N content and artificial diets with low protein and high carbohydrate content. Plant N content was lowest and locust abundance highest in heavily livestock-grazed fields where soils were N-depleted, likely due to enhanced erosion. These results suggest that heavy livestock grazing and consequent steppe degradation in the Eurasian grassland promote outbreaks of this locust by reducing plant protein content.

Plant nitrogen (N) content has long been linked to the performance and dynamics of herbivorous insects (*1*). Most nitrogen in plants is in the form of protein, and current paradigms generally assume that increased plant N content will enhance herbivore performance by relieving any constraints on growth and reproduction due to limitations of available protein (*2*). Because plants in many terrestrial ecosystems are N-limited (*3*), it is often assumed that primary consumers would be as well (*2*). However, studies performed on the basis of the geometric framework (*4*) have shown that many animals have specific dietary intake targets for protein, carbohydrates, and other nutrients. Interestingly, such studies have found that excess protein decreased life span in fruit flies (*5*) and that herbivores and omnivores (including humans) will overeat carbohydrates but are unlikely to overeat protein (*6*). Thus, animals may sometimes be impaired by ingesting excess protein (*7*). Indeed, the Australian plague locust, *Chortoicetes terminifera* (Acrididae: Oedipodinae), grew slowly on one host plant because it obtained excess protein relative to carbohydrate (*8*). Given that humans have substantially altered ecosystem N cycling through fossil fuel combustion (*9*), agricultural fertilizer application (*9*), and domesticated animal production (*10*), they may also affect insect dynamics in unexpected ways by altering plant nitrogen supplies. Understanding these anthropogenic impacts is critical to developing sustainable land management practices that minimize economically damaging insect outbreaks.

Oedaleus asiaticus (Acrididae: Oedipodinae) is a nonmodel [e.g., (*11*)], economically damaging locust of the north Asian steppe (*12, 13*), part of the largest grasslands in the world (Fig. 1A). We examined the effects of increases in the N content of host plants due to fertilization inputs of 175 kg N ha^{-1} year^{-1} on growth and viability of this locust in both laboratory and field cage experiments (*14*). This level of N addition is similar to the fertilization rates of most crops (*15*). We then used artificial diets varying in their protein:carbohydrate ratio to assess effects on dietary preference and growth rates.

In contrast to the existing paradigm of limitation of insects by low content of plant protein, our results show that N fertilization and high-protein artificial diets can have consistent negative effects on the performance of the locust. Survival decreased strongly with N fertilization in the field (Fig. 1B). The decreased survival of *O. asiaticus* in N-fertilized field plots could have been due to many possible consequences of fertilization, such as changes in plant structure or chemical content, species composition, predators, or microclimate. However, results from lab experiments that controlled for these factors indicated that growth rate, size, and development rate were all reduced when locusts were fed N-fertilized plants (Fig. 1C), indicating that the field survival results were due to effects associated with plant N status. Furthermore, when offered complementary artificial diets [e.g., (*16*)], locusts selectively consumed a protein:carbohydrate ratio of 0.5, which most correlated with maximal survival (Fig. 1, D and E). Performance (growth rate × survival) was significantly reduced when locusts were confined to artificial diets with a protein:carbohydrate ratio above 1:1 (Fig. 1E), consistent with the hypothesis that the mechanism by which N fertilization reduces survival of this locust in the field is elevation of plant protein content.

In addition, plant N content was closely associated with the relative amounts of different food plants consumed when locusts were offered a palette of the six most common local plants collected from unfertilized plots (five grasses and one sedge) (Fig. 2A). Indeed, contrary to expectations from protein-limitation paradigms, *Stipa grandis*, the grass with the lowest N content, was the most highly consumed over 36 hours [a period long enough for locusts to take multiple meals and regulate nutrient intake on the basis of internal conditions (*17*)]. Direct behavioral observations confirm that low-N *S. grandis* was the most highly consumed plant under field conditions (*18*).

We compared food selection over 8 hours between N-fertilized and unfertilized *S. grandis*, using dried, ground leaves to control for toughness and water content (*8, 19*). Locusts strongly favored unfertilized *S. grandis* leaves over *S. grandis*

[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA. [2]State Key Laboratory of Integrated Pest Management, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

*To whom correspondence should be addressed. E-mail: arianne.cease@asu.edu (A.J.C); jon.harrison@asu.edu (J.F.H); james.elser@asu.edu (J.J.E.); lkang@ioz.ac.cn (L.K.)

collected from N-fertilized plots (Table 1). N fertilization increased the protein content of *S. grandis* (Table 2), suggesting that the negative effects of N fertilization are due to changes in the protein:carbohydrate ratio away from the intake target of *O. asiaticus* (*20*), although it is also possible that N fertilization increased the concentration of N-rich alkaloids or other allelochemicals (*21*).

In Inner Mongolia, a primary outcome of heavy livestock grazing is the loss of topsoil and organic N due to erosion, resulting in plants with a low N content (*10*). Grazing also results in a shift in the balance between *S. grandis* and *Leymus chinensis*, with *S. grandis* becoming more prevalent in heavily grazed fields (*13*). [Note, however, that there was always a substantial biomass of each species available across our study sites and field cages (table S1).] Together, these grasses comprise roughly 80% of the above-ground biomass in the Inner Mongolian grasslands (*22*); thus, a large locust outbreak must depend on one or, more likely, both of these grasses during its development.

Consistent with a population-level impact of sheep grazing on locust abundance via effects on plant N content, locust density was highest in heavily grazed pastures dominated by plants with the lowest N content (Fig. 2B). Furthermore, locusts preferred low-N *S. grandis* collected from heavily grazed pastures over *S. grandis* from ungrazed plots (on the basis of paired-choice comparisons involving either fresh leaves or dried, ground leaves, to control for toughness and water content) (Table 1). Although the field measurements of locust abundance did not control for other possible effects of livestock grazing [e.g., shifts in predator communities, microclimate, sward structure, plant secondary chemicals, and landscape patchiness (*23*)], these data are consistent with the hypothesis that the high densities of *O. asiaticus* on the heavily grazed plots are due to this locust's preference for low-N plants and its improved growth and survival on such plants.

The hypothesis that low plant N can stimulate locust outbreaks is also supported by observations that *O. asiaticus* population explosions occur exclusively in pastures heavily grazed by livestock (*24*). Our data are in accordance with work showing that some North American Acrididae species (grasshoppers and locusts) also prefer low-N plants, likely due to their long-term adaptation to N-limited grassland environments (*25*). There is mounting evidence that N excess, rather than N limitation, is a critical nutritional factor regulating plant-insect interactions, especially for grasshoppers (*8*, *26*) and other herbivores that have evolved to exploit a relatively poor autotroph nutrient base in nature.

Our results fit well with the emerging paradigm that related animal species can vary dramatically in intake targets and performance responses to dietary variation. Seven cogeneric grasshopper species from one community were tested and all but two had unique target intake ratios, which suggests that each species may fill a unique nutritional niche and that species with extreme intake targets might be most sensitive to environmental variation (*27*). The lowest protein:carbohydrate intake target observed was 0.7, similar to what has been reported for the migratory locust (*L. migratoria*), which shares a subfamily with *Oedaleus* (*28*). We found that *O. asiaticus* had a protein:carbohydrate intake target of 0.5 (Fig. 1D), lower than any grasshopper previously studied (*21*). This finding may explain why dramatic, damaging population outbreaks of *O. asiaticus* occur in association with changes in land management (in this case, grazing intensity).

Our results strongly suggest that heavy livestock grazing promotes outbreaks of this locust by shifting plant nutrient content toward lower N conditions favorable to *O. asiaticus*. Although the precise physiological mechanisms by which high plant N exerts negative effects on this locust in the field remain to be elucidated, data from our artificial diet studies strongly suggest that protein
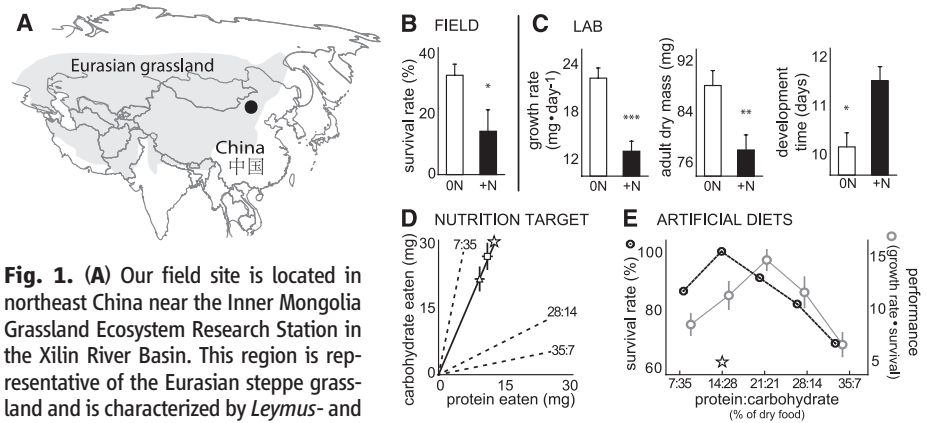

Fig. 1. (**A**) Our field site is located in northeast China near the Inner Mongolia Grassland Ecosystem Research Station in the Xilin River Basin. This region is representative of the Eurasian steppe grassland and is characterized by *Leymus*- and *Stipa*-dominated plant communities (*22*). Rapid steppe degradation (*29*) has led to reduced biodiversity, decreased productivity and, in some cases, desertification (*30*), likely due to anthropogenic factors, especially livestock grazing (*31*). N fertilization of field plots negatively affected *O. asiaticus*, (**B**) reducing survival in field cages and (**C**) growth, size, and development rates when reared in the laboratory (on *L. chinensis* from these plots). All comparisons were made using Student's *t* tests; data for proportion surviving in field cages were arcsine transformed before analysis (*$P < 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$). In artificial diet studies of field-collected animals, (**D**) locusts given pairs of diets complementary in their protein:carbohydrate ratios—7:35 and 35:7 (square) or 7:35 and 28:14 (triangle)—ate similar amounts of carbohydrate and protein (multivariate analysis of covariance: Wilks' lambda = 0.95, $F_{2,29} = 0.72$, $P = 0.50$; initial locust mass covariate means, 244 mg). Locusts fed nonrandomly to achieve a target protein:carbohydrate intake of ~0.5. (**E**) Locusts had 100% survival when fed artificial diets with a 0.5 protein:carbohydrate ratio, the same ratio they selected when given a choice. Survival rate was lowest (68%) in the highest protein diet. Overall performance (i.e., growth rate × survival) decreased above and below a protein:carbohydrate ratio of 1:1 (analysis of covariance: $F_{4,68} = 8.85$, $P < 0.001$; initial locust mass covariate means, 136 mg).

Fig. 2. (**A**) The percentage of consumption of each plant species (see SOM for how this was calculated) was negatively related to the percentage of N of that species (N was measured in leaf blades). Locusts were concurrently offered leaf blades from each of the six most common plants for 36 hours. 1, *S. grandis*; 2, *Carex korshinskyi*; 3, *Agropyron cristatum*; 4, *Cleistogenes squarrosa*; 5, *L. chinensis*; 6, *Achnatherum sibiricum*. Darkened squares are the two most common plants, each responsible for ~40% of aboveground biomass. Remaining species make up <5% each of aboveground biomass. (**B**) *O. asiaticus* was most abundant in heavily grazed fields where the N content of *S. grandis* was lowest during an outbreak year (2009); collected from large (>20 ha) and adjacent pastures [analysis of variance (ANOVA): $F_{3,16} = 64.95$, $P < 0.001$]. The ungrazed field has been fenced and protected since 1979; adjacent fields have been grazed at relatively constant levels for >5 years. Grazing level was determined by

differences in ground cover and aboveground biomass [e.g., (*12*)]. Each observation represents the number of *O. asiaticus* collected in sweep-net samples from a given pasture and the percentage of N of a concurrently collected composite of above ground *S. grandis* leaves from a 1-m² quadrat (inset) (ANOVA: $F_{3,12} = 14.81$, $P < 0.001$). Letters indicate differences using Scheffe's post hoc comparison tests.
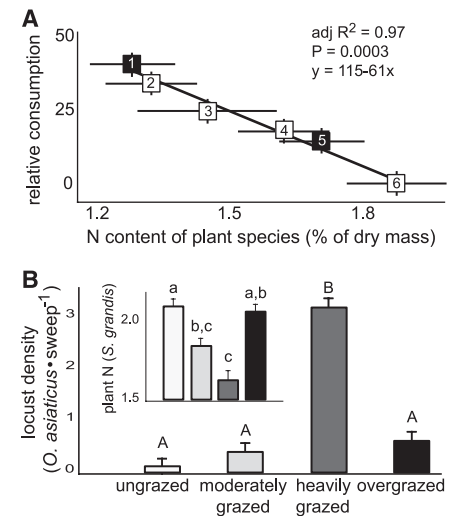
Table 1. Nitrogen enrichment of field plots decreased the palatability of *S. grandis* (the preferred host plant), and heavy grazing increased palatability in paired-choice tests using either dried/ground (8-hour assay) or fresh leaves (single-meal assay, average percentage of leaf area consumed).

Treatment (*S. grandis*)	Number of wins	Amount consumed	Chi- squared *P*
		Ground leaf	
Unfertilized	18	13.8 ± 2*	<0.01
Fertilized	7	8.6 ± 2*	
Heavily grazed	9	4.1 ± 1*	0.01
Ungrazed	3	2.9 ± 1*	
		Fresh leaf	
Heavily grazed	8	28 ± 7†	0.03
Ungrazed	3	15 ± 5†	

Table 2. Nitrogen enrichment of field plots increased the N content (percentage of dry mass) and protein content (percentage of dry mass) of *S. grandis* and *L. chinensis*. All comparisons were analyzed using Student's *t* tests after arcsine transformation of proportional data.

Nutrient	Unfertilized	N-fertilized	*P*	N	Heavily grazed	Ungrazed	*P*	N
			S. grandis					
% C	46 ± 0.8	46 ± 0.8	0.77	24	47 ± 0.2	44 ± 2	0.12	9
% N	1.4 ± 0.05	2.4 ± 0.06	<0.001	24	1.7 ± 0.05	2.1 ± 0.04	<0.001	9
% protein	4.3 ± 0.5	11.1 ± 1.8	<0.01	24				
Protein/N ratio	3.1 ± 0.4	4.6 ± 0.7	0.10	24				
			L. chinensis					
% C	46 ± 1	46 ± 0.8	0.99	24	46 ± 0.3	46 ± 0.3	0.18	8
% N	1.8 ± 0.08	2.9 ± 0.1	<0.001	24	2.4 ± 0.09	2.7 ± 0.03	0.12	8
% protein	8.7 ± 1	11.9 ± 1	0.03	23				
Protein/N ratio	4.9 ± 0.5	4.2 ± 0.4	0.37	23				

excess is playing a role. Furthermore, these findings reinforce the realization that differential responses of herbivore species to plant nutrient content can structure herbivore communities (*27*), providing new insights that may improve livestock and fertilization management strategies to limit the occurrence of economically damaging locust outbreaks.

References and Notes

1. W. J. Mattson Jr., *Annu. Rev. Ecol. Syst.* **11**, 119 (1980).
2. T. White, *The Inadequate Environment: Nitrogen and the Abundance of Animals* (Springer-Verlag Berlin, 1993).
3. J. J. Elser *et al.*, *Ecol. Lett.* **10**, 1135 (2007).
4. D. Raubenheimer, S. J. Simpson, *Anim. Behav.* **45**, 953 (1993).
5. K. P. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2498 (2008).
6. S. J. Simpson, D. Raubenheimer, *Aging* **1**, 875 (2009).
7. E. A. Bernays, in *Nitrogen as an Ecological Factor*, J. A. Lee, S. McNeill, I. H. Ronson, Eds. (Blackwell, Oxford, 1983), pp. 321–344.
8. F. J. Clissold, G. D. Sanson, J. Read, *J. Anim. Ecol.* **75**, 1000 (2006).
9. J. Galloway *et al.*, *Biogeochemistry* **70**, 153 (2004).
10. L. Wu, N. He, Y. Wang, X. Han, *J. Environ. Qual.* **37**, 663 (2008).
11. H. Song, *Psyche* **2011**, 1 (2011).
12. A. J. Cease, S. Hao, L. Kang, J. J. Elser, J. F. Harrison, *J. Insect Physiol.* **56**, 926 (2010).
13. L. Kang, X. G. Han, Z. B. Zhang, O. J. Sun, *Philos. Trans. R. Soc. London Ser. B* **362**, 997 (2007).
14. Materials and methods are available as supporting material on *Science* Online.
15. IFA/IFDC/FAO, *Fertilizer Use by Crop*. (Food and Agriculture Organization of the United Nations, Rome, ed. 5, 2006).
16. D. Raubenheimer, S. Simpson, *Entomol. Exp. Appl.* **91**, 67 (1999).
17. D. Raubenheimer, S. Jones, *Anim. Behav.* **71**, 1253 (2006).
18. D. F. B. Flynn, thesis, Columbia University, New York, (2011).
19. F. J. Clissold, G. D. Sanson, J. Read, S. J. Simpson, *Ecology* **90**, 3393 (2009).
20. D. Raubenheimer, S. J. Simpson, *J. Exp. Biol.* **206**, 1669 (2003).
21. S. T. Behmer, *Annu. Rev. Entomol.* **54**, 165 (2009).
22. Z. Chen, S. Wang, *Typical Steppe Ecosystems of China* (Science Press, Beijing, 2000).
23. D. H. Branson, A. Joern, G. A. Sword, *Bioscience* **56**, 743 (2006).
24. L. Kang, Y. L. Chen, *Insect Sci.* **2**, 265 (1995).
25. A. Joern, S. T. Behmer, *Oecologia* **112**, 201 (1997).
26. D. Berner, W. U. Blanckenhorn, C. Korner, *Oikos* **111**, 525 (2005).
27. S. T. Behmer, A. Joern, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 1977 (2008).
28. S. Simpson, D. Raubenheimer, *Philos. Trans. R. Soc. London Ser. B* **342**, 381 (1993).
29. C. Tong, J. Wu, S. Yong, J. Yang, W. Yong, *J. Arid Environ.* **59**, 133 (2004).
30. J. Wu, C. Overton, *Bull. Ecol. Soc. Am.* **83**, 189 (2002).
31. J. Han *et al.*, *Rangeland J.* **30**, 233 (2008).

Depth Perception from Image Defocus in a Jumping Spider

Takashi Nagata,[1]* Mitsumasa Koyanagi,[1]† Hisao Tsukamoto,[1] Shinjiro Saeki,[2] Kunio Isono,[2] Yoshinori Shichida,[3] Fumio Tokunaga,[4] Michiyo Kinoshita,[5] Kentaro Arikawa,[5] Akihisa Terakita[1]†

The principal eyes of jumping spiders have a unique retina with four tiered photoreceptor layers, on each of which light of different wavelengths is focused by a lens with appreciable chromatic aberration. We found that all photoreceptors in both the deepest and second-deepest layers contain a green-sensitive visual pigment, although green light is only focused on the deepest layer. This mismatch indicates that the second-deepest layer always receives defocused images, which contain depth information of the scene in optical theory. Behavioral experiments revealed that depth perception in the spider was affected by the wavelength of the illuminating light, which affects the amount of defocus in the images resulting from chromatic aberration. Therefore, we propose a depth perception mechanism based on how much the retinal image is defocused.

Visual systems that accurately and reliably judge distance or depth are valuable. A wide variety of animals, including humans, perform this task with binocular stereoscopic depth perception (*1*). Two types of monocular depth cues also provide absolute depth perception in some animals: accommodation (i.e., focal adjustment) in chameleons and other vertebrates (*2, 3*)