Read this page, paying particular attention to the highlighted paragraph. Note it's structure: the topic sentence, supporting sentences and last sentences. Afterwards, see the commented version on the next page.

Evolutionary ecology of pungency in wild chilies

Joshua J. Tewksbury*†, Karen M. Reagan*, Noelle J. Machnicki*, Tomás A. Carlo*, David C. Haak*, Alejandra Lorena Calderón Peñaloza‡, and Douglas J. Levey§

*Department of Biology, University of Washington, Box 351800, 24 Kincaid Hall, Seattle, WA 98195-1800; ‡Carrera de Biología, Facultad Ciencias Agrícolas, Universidad Autónoma Gabriel René Moreno, Vallecito Km 9 Norte, Santa Cruz de la Sierra, Bolivia; and §Department of Zoology, PO 118525, University of Florida, Gainesville, FL 32611-8525

Edited by May R. Berenbaum, University of Illinois at Urbana–Champaign, Urbana, IL, and approved May 16, 2008 (received for review March 18, 2008)

The primary function of fruit is to attract animals that disperse viable seeds, but the nutritional rewards that attract beneficial consumers also attract consumers that kill seeds instead of dispersing them. Many of these unwanted consumers are microbes, and microbial defense is commonly invoked to explain the bitter, distasteful, occasionally toxic chemicals found in many ripe fruits. This explanation has been criticized, however, due to a lack of evidence that microbial consumers influence fruit chemistry in wild populations. In the present study, we use wild chilies to show that chemical defense of ripe fruit reflects variation in the risk of microbial attack. Capsaicinoids are the chemicals responsible for the well known pungency of chili fruits. *Capsicum chacoense* is naturally polymorphic for the production of capsaicinoids and displays geographic variation in the proportion of individual plants in a population that produce capsaicinoids. We show that this variation is directly linked to variation in the damage caused by a fungal pathogen of chili seeds. We find that *Fusarium* fungus is the primary cause of predispersal chili seed mortality, and we experimentally demonstrate that capsaicinoids protect chili seeds from *Fusarium*. Further, foraging by hemipteran insects facilitates the entry of *Fusarium* into fruits, and we show that variation in hemipteran foraging pressure among chili populations predicts the proportion of plants in a population producing capsaicinoids. These results suggest that the pungency in chilies may be an adaptive response to selection by a microbial pathogen, supporting the influence of microbial consumers on fruit chemistry.

directed deterrence | frugivory | fruit chemistry | secondary metabolite | *Capsicum chacoense*

The evolution of fruit, a reward for animal dispersal of seeds, is a commonly cited example of a key innovation in the radiation of angiosperms (1–3). However, the nutritional qualities of fruit pulp that are responsible for attracting beneficial dispersers also attract consumers that are detrimental to plant fitness. These consumers range from vertebrate and invertebrate seed predators to microbial consumers of fruits and seeds that reduce the likelihood of dispersal and the viability of seeds (4). Fruit chemistry is commonly thought to mediate these interactions, either by deterring seed predators (4–6) or reducing microbial attack of fruits and seeds (4, 7, 8). These mechanisms are not mutually exclusive, but chemicals that deter fruit consumption often affect a wide range of species (7, 9), and defensive chemistry in ripe fruit must be sufficiently targeted toward detrimental organisms to allow consumption by vertebrate seed dispersers. Fruit secondary compounds that deter microbial consumers without reducing seed dispersal by vertebrates are thought to be far more plausible than secondary compounds that selectively deter vertebrate predators (7), because microbial fruit consumers are uniformly negative in their impacts on plant fitness (4) and are farther removed in their morphology, physiology, and mode of consumption from vertebrate seed dispersers than are other unwanted consumers (4, 7).

Microbial deterrence is thus a primary hypothesis explaining the presence of noxious, bitter, and sometimes toxic chemicals in many ripe fruits; the negative effects these chemicals often have on vertebrate dispersers are assumed to be balanced by the benefits of deterring microbial consumers. Unfortunately, this hypothesis remains largely untested, because no work to date has shown that variance in microbial pathogen pressure is related to variance in the chemistry of ripe fruits in wild populations. A strong test would require a species in which fruit chemistry is well known, likely to protect against microbial pathogens, unique to the fruit, and highly variable. The most famous plants with these qualities are chilies (genus *Capsicum*). Chilies were one of the first plants domesticated in the New World (10), and they are now consumed by one in four humans daily (11), largely because of the pungency produced by capsaicinoids. Capsaicinoids are well characterized (9) and broadly antimicrobial (12–14). In fact, early humans likely selected chilies for use and domestication expressly because of their antimicrobial properties (12, 15). Finally, because capsaicinoids are found only within the fruit of *Capsicum* species and their concentrations increase during fruit ripening (16), the function of these chemicals is likely restricted in the fruit itself, not attributable to alternative functions in other parts of the plant (17).

Chilies thus provide an exceptionally clear window into the function of fruit chemistry, and our recent rediscovery of a polymorphism for capsaicinoid production in wild populations of multiple chili species (18) provides the variability we need to explicitly examine the function of these chemicals in wild populations. We have studied this polymorphism most intensively in *Capsicum chacoense* Hunz., which is native to the Chaco region of Bolivia, Argentina, and Paraguay (19). In polymorphic populations, *C. chacoense* plants producing fruits that contain capsaicinoids grow alongside plants with fruits that are nutritionally similar (20) but completely lack capsaicinoids (18) [see supporting information (SI)]. In addition, the proportion of plants producing capsaicinoids varies widely among populations. At the southwestern end of our 300-km-long study area in southeastern Bolivia, the polymorphism is virtually absent; most populations contain only pungent plants. To the north and east of this area, nonpungent plants gradually increase in frequency, until >70% of individuals lack capsaicinoids, and the few plants that do produce pungent fruit have capsaicinoid concentrations barely one-third the level found in completely pungent populations (18).

We use this geographic gradient as a tool to study the impact of microbial pathogens on fruit chemistry, and we made the following predictions: (*i*) Microbial fruit pathogens will have a large negative impact on nonpungent chilies, (*ii*) capsaicinoids will reduce microbial damage to chili fruits and seeds, and (*iii*) among populations, the proportion of plants producing capsaicinoids will increase as the intensity of microbial attack increases.

Author contributions: J.J.T., K.M.R., N.J.M., T.A.C., D.C.H., and D.J.L. designed research; J.J.T., K.M.R., N.J.M., T.A.C., A.L.C.P., and D.J.L. performed research; J.J.T. analyzed data; and J.J.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

†To whom correspondence should be addressed. E-mail: tewksjj@u.washington.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0802691105/DCSupplemental.

Evolutionary ecology of pungency in wild chilies

Joshua J. Tewksbury*†, Karen M. Reagan*, Noelle J. Machnicki*, Tomás A. Carlo*, David C. Haak*, Alejandra Lorena Calderón Peñaloza‡, and Douglas J. Levey§

*Department of Biology, University of Washington, Box 351800, 24 Kincaid Hall, Seattle, WA 98195-1800; ‡Carrera de Biología, Facultad Ciencias Agrícolas, Universidad Autónoma Gabriel René Moreno, Vallecito Km 9 Norte, Santa Cruz de la Sierra, Bolivia; and §Department of Zoology, PO 118525, University of Florida, Gainesville, FL 32611-8525

Edited by May R. Berenbaum, University of Illinois at Urbana–Champaign, Urbana, IL, and approved May 16, 2008 (received for review March 18, 2008)

The primary function of fruit is to attract animals that disperse viable seeds, but the nutritional rewards that attract beneficial consumers also attract consumers that kill seeds instead of dispersing them. Many of these unwanted consumers are microbes, and microbial defense is commonly invoked to explain the bitter, ound in many ripe fruits. wever, due to a lack of ce fruit chemistry in wild wild chilies to show that variation in the risk of hemicals responsible for *Capsicum chacoense* is n of capsaicinoids and rtion of individual plants ids. We show that this variation is directly linked to variation in the damage caused by a fungal pathogen of chili seeds. We find that *Fusarium* fungus is the primary cause of predispersal chili seed mortality, and we experimentally demonstrate that capsaicinoids protect chili seeds from *Fusarium*. Further, foraging by hemipteran insects facilitates the en t variation in he ns predicts the pr capsaicinoids. Th s may be an ad gen, supporting ing hemistry.

The topic sentence makes the paragraph's idea clear: the chilies' polymorphism has the necessary variability to test their hypothesis. This is the idea that will be developed in the following sentences.

The supporting sentences in the middle provide descriptions of the study area and the chilies' variability there. They expand on the topic sentence, going into more detail as they describe the specific species and area. The authors provide several references here. The transitional phrase "in addition" is used to link sentences.

dir metabolite | *Ca*

T rsal of seeds, vation in the ra ritional qual- iti ing beneficial dis ental to plant fitness. These consumers range from vertebrate and invertebrate seed predators to microbial consumers of fruits and seeds that reduce the likelihood of dispersal and the viability of seeds (4). Fruit chemistry is commonly thought to mediate these interactions, either by deterring seed predators (4–6) or reducing microbia hanisms are not uit consumptio 9), and defensiv targeted toward by vertebrate se at deter microbia by vertebrates a condary compou (7), because m in their impacts in their morphology, physiology, and mode of consumption from vertebrate seed dispersers than are other unwanted consumers (4, 7).

The last two sentences wrap up the description of the area's variability using more detailed facts to describe it clearly. These sentences back up the paragraph's claim stated in the topic sentence, and thus conclude the paragraph.

Microbial deterrence is thus a primary hypothesis explaining the presence of noxious, bitter, and sometimes toxic chemicals in many ripe fruits; the negative effects these chemicals often have on vertebrate dispersers are assumed to be balanced by the benefits of deterring microbial consumers. Unfortunately, this hypothesis remains largely untested, because no work to date has shown that variance in microbial pathogen pressure is related to variance in the chemistry of ripe fruits in wild populations. A strong test would require a species in which fruit chemistry is well known, likely to protect against microbial pathogens, unique to the fruit, and highly variable. The most qualities are chilies (genus *Capsicum*) first plants domesticated in the New now consumed by one in four humans of the pungency produced by capsaic well characterized (9) and broadly anti early humans likely selected chilies fo expressly because of their antimicrob Finally, because capsaicinoids are foun *Capsicum* species and their concentrat ripening (16), the function of these che in the fruit itself, not attributable to alternative functions in other parts of the plant (17).

The word "thus" refers back to the point made in the previous paragraph, creating a logical connection- and a nice transition - between them.

Chilies thus provide an exceptionally clear window into the function of fruit chemistry, and our recent rediscovery of a polymorphism for capsaicinoid production in wild populations of multiple chili species (18) provides the variability we need to explicitly examine the function of these chemicals in wild populations. We have studied this polymorphism most intensively in *Capsicum chacoense* Hunz., which is native to the Chaco region of Bolivia, Argentina, and Paraguay (19). In polymorphic populations, *C. chacoense* plants producing fruits that contain capsaicinoids grow alongside plants with fruits that are nutritionally similar (20) but completely lack capsaicinoids (18) [see supporting information (SI)]. In addition, the proportion of plants producing capsaicinoids varies widely among populations. At the southwestern end of our 300-km-long study area in southeastern Bolivia, the polymorphism is virtually absent; most populations contain only pungent plants. To the north and east of this area, nonpungent plants gradually increase in frequency, until >70% of individuals lack capsaicinoids, and the few plants that do produce pungent fruit have capsaicinoid concentrations barely one-third the level found in completely pungent populations (18).

We use this geographic gradient as a tool to study the impact of microbial pathogens on fruit chemistry, and we made the following predictions: (*i*) Microbial fruit pathogens will have a large negative impact on nonpungent chilies, (*ii*) capsaicinoids will reduce microbial damage to chili fruits and seeds, and (*iii*) among populations, the proportion of plants producing capsaicinoids will increase as the intensity of microbial attack increases.

The first sentence of the following paragraph refers directly to the last two sentences, creating a smooth transition between paragraphs.

Author contributions: J.J.T., K.M.R., N.J.M., T.A.C., D. J.J.T., K.M.R., N.J.M., T.A.C., A.L.C.P., and D.J.L. perfor and J.J.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

†To whom correspondence should be addressed. E-ma

This article contains supporting information online 0802691105/DCSupplemental.