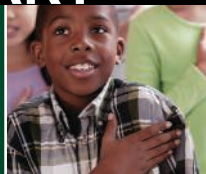


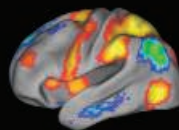
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## LETTERS

edited by Etta Kavanagh

### A Debate Over Iraqi Death Estimates

JOHN BOHANNON'S ARTICLE "IRAQI DEATH ESTIMATES CALLED TOO high; methods faulted" (News of the Week, 20 Oct., p. 396) contains several errors that require comment.

Bohannon fails to appreciate that cluster sampling is a random sampling method. Sampling for our study was designed to give all households an equal chance of being included. In this multistage cluster sampling, random selections were made at several levels ending with the "start" house being randomly chosen. From there, the house with the nearest front door was sampled until 39 consecutive houses were selected. This usually involved a chain of houses extending into two or three adjacent streets. Using two teams of two persons each, 40 houses could be surveyed in one day. Of our 47 clusters, 13 or 28% were rural, approximating the UN estimates for the rural population of Iraq.

Bohannon states that Gilbert Burnham did not know exactly how the Iraqi team conducted its survey. The text sent to Bohannon, which he fails to cite, said, "As far as selection of the start houses, in areas where there were residential streets that did not cross the main avenues in the area selected, these were included in the random street selection process, in an effort to reduce the selection bias that more busy streets would have." In no place does our *Lancet* paper say that the survey team avoided small back alleys. The methods section of the paper was modified with the suggestions of peer

reviewers and the editorial staff. At no time did Burnham describe it to Bohannon as "oversimplified."

Those who work in conflict situations know that checkpoints often scrutinize written materials carried by those stopped, and their purpose may be questioned. Unique identifiers, such as neighborhoods, streets, and houses, would pose a risk not only to those in survey locations, but also to the survey teams. Protection of human subjects is always paramount in field research. Not including unique identifiers was specified in the approval the study received from the Johns Hopkins Bloomberg School of Public Health Committee on Human Research. At no time did the teams "destroy" details, as Bohannon contends. Not recording unique identifiers does not compromise the validity of our results.

Concerning mortality estimates, Michael Spagat may be content, as Bohannon claims, with mortality data collected barely 1 year into an escalating 3.5-year war. Others might not find these so helpful.

**GILBERT BURNHAM AND LES ROBERTS**

Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA.



An Iraqi woman collapses after learning of the death of a relative in a bomb attack on a police car.

### Response

I DO APPRECIATE THAT CLUSTER SAMPLING relies on random samples. It is indeed the very bone of contention. "Sampling for our study was designed to give all households an equal chance of being included," Burnham and Roberts write. But according to their methods as published in *The Lancet*, that is not the case.

My article reports the concerns of Sean Gourley and Neil Johnson, who point out that the starting house was always on a street "randomly selected from a list of residential streets crossing the main street." This excludes all the smaller streets—including back alleys—that do not cross a main street. Maps of Iraqi cities, freely available at [www.earth.google.com](http://www.earth.google.com), show that many residential areas would be excluded by this

survey protocol. People living in those underrepresented households, Gourley and Johnson argue, are less likely to be exposed to the violence—car bombs, drive-by shootings, airstrikes—that accounts for most of the reported deaths.

When I asked Burnham by e-mail about this possible source of bias, he replied that "in areas where there were residential streets that did not cross the main avenues in the area selected, these were included in the random street selection process, in an effort to reduce the selection bias that more busy streets would have." When I asked him why the published methods leave out this wiggle room, he replied that "in trying to shorten the paper from its original very large size, this bit got chopped, unfortunately." I used the term "oversimplified" to describe this discrepancy.

I stated that "the details about neighborhoods surveyed were destroyed." The details in question are the "scraps" of paper on which streets and addresses were written to "randomly" choose households, and as Burnham and Roberts explained to me, that record has indeed been destroyed. I appreciate the difficulty of conducting a study in a combat zone and also the researchers' desire to protect the survey team and respondents. At the same time, scientists concerned about the true number of Iraqi casualties want to know which method was used to select households and whether sample bias can explain the high number of violent deaths reported by Burnham *et al.* But without a clear and explicit methodology or raw data to independently examine, it is impossible to know.

**JOHN BOHANNON**

## A Nonprotein Amino Acid and Neurodegeneration

RESEARCH ON  $\beta$ -METHYLAMINO-L-ALANINE (BMAA) and neurodegenerative disease among the Chamorro people of Guam lost momentum when M. W. Duncan reported BMAA levels in washed cycad flour far lower than those reported to generate acute neurotoxicity in primates (1, 2). We hypothesized that the Chamorros may be exposed to increased levels of cycad neurotoxins, including BMAA, when they eat flying foxes and other animals that forage on cycad seeds (3). Two new

findings—selective neurotoxicity of BMAA to motor neurons at low concentrations (4) and alternative inputs of BMAA in the Chamorro diet (5)—have brought renewed attention to BMAA. M. W. Duncan and A. M.

Marini's Letter "Debating the cause of a neurological disorder" (22 Sept., p. 1737) needs clarification, as the authors may have been unaware of recent literature that supports the link between BMAA and neurological disease.

Their suggestion that BMAA "is not very neurotoxic" needs updating in light of evidence that 30  $\mu$ M BMAA selectively kills motor neurons (4). Duncan and Marini express concern about the three flying fox specimens analyzed in our 2003 paper (6), but we subsequently reported BMAA in an additional 21 specimens (7). They question the specificity of the assay we used, but 6-aminoquinolyl-*N*-hydroxy-succinimidyl carbamate, developed as a stable high-performance liquid chromatography fluorescent tag for hospital analysis of amino acids (8, 9), is more reliable than the less modern methods used by Montine *et al.* (10).

Questions about Chamorro consumption of flying foxes ignore evidence that hunting contributed to significant declines in flying fox populations (11). Over 220,000 dead flying foxes were imported within a 15-year period to meet resultant consumer demand (12). We have also found that high levels of BMAA occur

in protein fractions of cycad flour (13), which updates Duncan's earlier report (2).

The discovery that BMAA is produced by diverse taxa of cyanobacteria opens the possibility of human exposure far from Guam (14). Our blinded analysis of BMAA in control and diseased tissues, however, does not prove causality. The real question is not whether BMAA is present, but whether exposure to BMAA can produce progressive neurodegeneration. That question deserves a second look.

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## Plants, RNAi, and the Nobel Prize

IN JENNIFER COUZIN'S RECENT PIECE ON THE Nobel Prize that was awarded to Andy Fire and Craig Mello, an anonymous RNA interference (RNAi) researcher was quoted as saying "plants got screwed" ("Method to silence genes earns loud praise," *News of the Week*, 6 Oct., p. 34). As an early participant in the plant RNA silencing field, I take exception with this view. I feel that the Nobel committee's decision to focus on the central role of double-stranded RNA (dsRNA) was quite appropriate; it was this specific discovery that broke an obscure field wide open and brought it to the attention of all biologists. The publication of RNAi (1) catalyzed new interactions between plant and animal geneticists that led directly to all kinds of discoveries about the mechanisms underlying and related to RNAi. The impact on biological research from understanding that dsRNA is a key intermediate in triggering RNAi has been huge. dsRNA is used as a tool to silence genes in a significant percentage of all papers on eucaryotic biology (for instance, "RNA interference" was mentioned in more than 20% of

all research articles published this year in the journal I edit, *The Plant Cell*, the leading primary research journal in plant biology). Of course, there were also many other very important discoveries in the RNAi field, by researchers working in plants, animals, and fungi, but none of them had the same catalytic impact on biology as did Fire and Mello's key insight and elegant experimentation. The Nobel committee decided to keep the award simple and straightforward for good reason.

The Nobel Prize is not really about making scientists famous—it is about making science interesting and accessible to the public. RNAi is a wonderful vehicle for communicating the importance and potential of basic research. Many more people will now understand the value of fundamental research because of the RNAi story, and that is fantastic news for all scientists.

Congratulations, Andy and Craig, and thank you for your tremendous contribution to science!

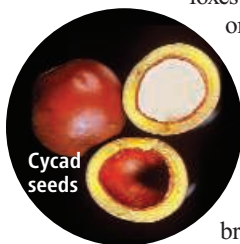
RICH JORGENSEN

Editor in Chief, *The Plant Cell*, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721-0036, USA.

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WE CONGRATULATE ANDREW FIRE AND CRAIG Mello on their Nobel Prize for the discovery of RNA interference (RNAi). Their experiments identified double-stranded RNA as a reliable trigger of gene silencing and attracted the interest of animal biologists. However, as plant scientists who were involved in some of the earliest work on gene silencing, we want to correct the impression conveyed in Jennifer Couzin's article "Method to silence genes earns loud praise" (*News of the Week*, 6 Oct., p. 34) that plant biologists made puzzling findings that were not tied together in any way. The general principle developed by plant biologists was "homology-dependent gene silencing," in which various combinations of "homologous" sequence interactions between DNA and/or RNA induce silencing at either the transcriptional or posttranscriptional level (1). This concept, which was novel at the time, underlies our current understanding of RNAi-mediated silencing pathways in both the cytoplasm and the nucleus. Epigenetic modifications induced by homologous sequence interactions, including RNA-directed DNA methylation (2), were identified in some of the earliest plant studies and paved the way for the discovery of RNAi-mediated heterochromatin formation in fission yeast. Connections between homology-dependent gene silencing and transposon control, virus resistance, and development were made early on by plant scientists (1, 3, 4) and are now considered, at least in part, to be



Cycad seeds

### Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web ([www.submit2science.org](http://www.submit2science.org)) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

RNAi-mediated processes. Double-stranded RNA as an intermediate in the silencing pathway in plants was proposed in models (4, 5) and directly tested in plant systems (6). Thus, plant research leading up to the discovery of RNAi in *C. elegans* cannot be regarded as a set

of diffuse observations that lacked a unifying theme, nor did plant scientists fail to recognize the broader implications of their work.

**MARJORI MATZKE AND ANTONIUS J. M. MATZKE**

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#### TECHNICAL COMMENT ABSTRACTS

### COMMENT ON Papers by Chong *et al.*, Nishio *et al.*, and Suri *et al.* on Diabetes Reversal in NOD Mice

**Denise L. Faustman, Simon D. Tran, Shohta Kodama, Beatrijs M. Lodde, Ildiko Szalayova, Sharon Key, Zsuzsanna Toth, Éva Mezey**

Chong *et al.*, Nishio *et al.*, and Suri *et al.* (Reports, 24 March 2006, pp. 1774, 1775, and 1778) confirmed that treating nonobese diabetic (NOD) mice with an immune adjuvant and semisyngenic spleen cells can reverse the disease but found that spleen cells did not contribute to the observed recovery of pancreatic islets. We show that islet regeneration predominately originates from endogenous cells but that introduced spleen cells can also contribute to islet recovery.

Full text at [www.sciencemag.org/cgi/content/full/314/5803/1243a](http://www.sciencemag.org/cgi/content/full/314/5803/1243a)

### RESPONSE TO COMMENT ON Chong *et al.* on Diabetes Reversal in NOD Mice

**Anita S. Chong, Jikun Shen, Jing Tao, Dengping Yin, Andrey Kuznetsov, Manami Hara, Louis H. Philipson**

We failed to detect transdifferentiation of spleen cells into  $\beta$  cells following diabetes reversal in nonobese diabetic (NOD) mice, thus contradicting a key finding of a 2003 report. We respond to Faustman *et al.* by justifying the use of mouse insulin promoter–green fluorescent protein transgenic mice as an appropriate system for detecting spleen-derived  $\beta$  cells in the islets of cured NOD mice.

Full text at [www.sciencemag.org/cgi/content/full/314/5803/1243b](http://www.sciencemag.org/cgi/content/full/314/5803/1243b)

### RESPONSE TO COMMENT ON Nishio *et al.* on Diabetes Reversal in NOD Mice

**Junko Nishio, Jason L. Gaglia, Stuart E. Turvey, Christopher Campbell, Christophe Benoist, Diane Mathis**

Contrary to previous findings, we found no significant differentiation of splenocytes into pancreatic islet cells

in nonobese diabetic (NOD) mice treated with an immune adjuvant and allogenic spleen cells. We show that our single-nucleotide polymorphism assay has the requisite sensitivity to support our contention. The experiments of Faustman *et al.* lack adequate controls, and we maintain that no evidence of islet regeneration has been presented.

Full text at [www.sciencemag.org/cgi/content/full/314/5803/1243c](http://www.sciencemag.org/cgi/content/full/314/5803/1243c)

### RESPONSE TO COMMENT ON Suri *et al.* on Diabetes Reversal in NOD Mice

**Anish Suri and Emil R. Unanue**

Faustman *et al.* present no new information to explain why three independent laboratories failed to reproduce their previous results implicating spleen cell transdifferentiation in the reversal of murine type 1 diabetes. Modulation of the immunological process in nonobese diabetic (NOD) mice has been accomplished by many laboratories using different protocols and does not represent a novel finding in their work.

Full text at [www.sciencemag.org/cgi/content/full/314/5803/1243d](http://www.sciencemag.org/cgi/content/full/314/5803/1243d)