the United States, annual research surveys have been conducted with a stratified random survey design [W. G. Doubleday and D. Rivard, Eds., *Bottom Trawl Surveys* (Department of Fisheries and Oceans, Ottawa, Canada, 1981)]. These surveys cover a range of depths from 50 to 400 m and are designed to provide unbiased estimates of abundance. Before 1971, a fixed location survey along line transects was used off the coasts of Newfoundland and Nova Scotia. These surveys were converted to the stratification scheme with the latitude, longitude, and depth of the tow. The earlier surveys were conducted primarily during the day, and, because skates are caught in substantially higher proportions at night, day catches would underestimate the true abundance (J. M. Casey and R. A. Myers, *Can. J. Fish. Aquat. Sci.*, in press). To account for this difference in diel catchability, we converted half of the catches before 1970 to night catches using a factor of 2.08. These surveys would then be comparable with those after 1970 in which day and night tows were conducted in roughly equal proportion. Biomass estimates were based on the stratified random design [W. J. Cochran, *Sampling Techniques* (Wiley, New York, 1977)]. Estimates of absolute abundance were obtained by dividing the biomass estimates by a factor of 0.15, if 10% of the skates in the path of the trawl are caught during the day [R. L. Edwards, in *The Future of the Fishing Industry of the United States*, D. Gilbert, Ed. (University of Washington, Seattle, WA, 1968), pp. 52–60] and 20% of the population would be caught at night. Neither of these conversions affects the observed biomass trends.

12. Because barndoor skates have only been caught in research surveys of Browns Bank and Georges Bank in the past 20 years, we examined the statistical power of detecting barndoor skate given a specific low abundance level. On St. Pierre Bank, 504 of 1075 research survey tows were conducted over the past 25 years at depths where, historically, barndoor skates were commonly found (200 to 400 m). We assume that the number n of barndoor skates caught in one tow has a negative binomial distribution, with a probability of $[\Gamma(k + n)/\Gamma(k)n!][\mu^n k^k/(\mu + k)^{k+n}]$, where μ is the mean catch per tow and the constant k is the inverse of aggregation. Then, with a theoretical low mean abundance of 500 individuals in a 7368-km^2 area (the tow sample area is 0.05 km^2, and the probability of catching a barndoor skate if encountered is 0.15) and $k = 0.5$, the probability of not detecting a barndoor skate in 504 tows is 0.77. Greater aggregation (that is, lower k) results in only small changes in this number. If 1000 individuals remained, the probability of not detecting a barndoor skate decreases to 0.6.
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16. The relation between the weight of the young at hatching, w, and the maximum estimate of fecundity is $10.9w^{-0.45}$ (*1*).
17. For the barndoor skate population to be self-sustaining, the survival to age x, l_x, must be sufficient such that $\Sigma l_x m_x = 1$, where m_x is the number of eggs developing into females from individuals at age x. If survival, P, is constant such that $l_x = P^x$, then $P^a m_o \Sigma_{i=0}^{\infty} P^i = 1$, where the survival to the age of maturity a is P^a and the fecundity in each year is m_o. Summing the geometric series, $P^a m_o[1/(1 - P)] = 1$. The instantaneous mortality rate is $\log(P)$. If the mortality of juveniles is twice that of adults, the instantaneous mortality required to drive the barndoor skate to extinction is 0.45.
18. This age estimate is based on the relation between somatic growth rate and temperature and a comparison of age at maturity of cod that occur at the same locations (*15*).
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21. We thank the Department of Fisheries and Oceans at St. John's and Halifax, Canada, and the National Marine Fisheries Service at Woods Hole, MA, for providing the data used in this analysis and B. Atkinson, K. Frank, J. E. Simon, M. Fogarty, S. Murawski, and J. Hutchings for helpful discussions. This research was initiated while R.A.M. was a visiting fellow at the Centre for Population Biology, Imperial College at Silwood Park.

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Regulation of a Transcription Factor Network Required for Differentiation and Metabolism

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Hepatocyte nuclear factors (HNFs) are a heterogeneous class of evolutionarily conserved transcription factors that are required for cellular differentiation and metabolism. Mutations in *HNF-1*α and *HNF-4*α genes impair insulin secretion and cause type 2 diabetes. Regulation of HNF-4/HNF-1 expression by *HNF-3*α and *HNF-3*β was studied in embryoid bodies in which one or both *HNF-3*α or *HNF-3*β alleles were inactivated. HNF-3β positively regulated the expression of *HNF-4α/HNF-1*α and their downstream targets, implicating a role in diabetes. HNF-3β was also necessary for expression of *HNF-3*α. In contrast, HNF-3α acts as a negative regulator of *HNF-4α/HNF-1*α demonstrating that HNF-3α and HNF-3β have antagonistic transcriptional regulatory functions in vivo. HNF-3α does not appear to act as a classic biochemical repressor but rather exerts its negative effect by competing for HNF-3 binding sites with the more efficient activator HNF-3β. In addition, the HNF-3α/HNF-3β ratio is modulated by the presence of insulin, providing evidence that the HNF network may have important roles in mediating the action of insulin.

Hepatocyte-specific gene expression is controlled primarily at a transcriptional level and relies on the activity of multiple transcription factors including HNF-1, CCAAT/enhancer binding protein (C/EBP), HNF-3, HNF-4, and HNF-6 (*1*). Our current understanding of transcriptional regulation by these HNFs has been derived primarily from analysis of promoter/enhancer elements of genes selectively expressed in cultured cells with transient transfections (*1*). Although these approaches have provided useful information about tissue-specific regulation of gene expression, it is important to note that such procedures do not measure transcriptional regulation within a native chromosomal context. This is significant, given that HNF-3 proteins have been shown to modify the nucleosomal organization of the albumin enhancer, a finding consistent with the structure of the HNF-3 DNA binding domain, which is highly similar to that of histone H5 (*2*). Analyses of specific promoter elements indicate that HNFs act in various combinations to direct cell-specific transcription during cellular differentiation (*3*). Targeted disruption of transcription factors in mice often results in only moderate reductions in target gene expression, which supports the hypothesis that transcription factors act cooperatively. In contrast to this, we have recently shown that disruption of *HNF-4*α drastically reduces the expression of numerous target genes, implying that it acts as a central regulator of tissue-specific gene expression (*4*). In addition, earlier studies had shown that HNF-4α also positively regulated expression of *HNF-1*α, defining a transcriptional hierarchy involved in maintaining the hepatic phenotype (*5*). We predicted that factors that control the expression of *HNF-4*α could have critical functions in directing cell differentiation. Moreover, the recent finding that mutations in the genes encoding HNF-4α and HNF-1α are responsible for two phenotypically indistinguishable forms of early-onset type 2 diabetes, MODY1 and MODY3, respectively, suggested that such upstream regulators of *HNF-4*α expression could also be necessary for normal pancreatic β cell function and metabolism (*6*).

Evolutionarily conserved HNF-3 binding

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sites have previously been identified in a portion of the HNF-4α promoter that is sufficient to drive tissue-specific expression of a transgene in mice (*7*). *HNF-3β* and *HNF-4α* null mice are embryonic lethal and therefore cannot be used for gene expression studies (*8*). To test whether HNF-3 regulated the expression of *HNF-4α*, we therefore generated *HNF-3α* and *HNF-3β* null embryonic stem (ES) cells. ES cells grown in suspension differentiate to form visceral endoderm (VE) that expresses *HNF-3α* and -3β, as well as *HNF-4α* and *HNF-1α* and their target genes. We have previously shown that the regulation of gene expression in the VE directly parallels that in the liver (*4*). The VE, therefore, provides a suitable system for studying transcriptional regulation of metabolism in the liver and pancreas because they all share expression of the HNF transcription factors (*9*). To study HNF-3–dependent gene expression, we generated *HNF-3α* and *HNF-3β* null ES cells. The strategy for disruption of *HNF-3β* in ES cells has been described (*8*). The *HNF-3α* gene was mutated by targeted deletion of the DNA binding domain and the COOH-terminal transcriptional transactivation domains by homologous recombination in ES cells (*10*). In addition, the *Escherichia coli LacZ* gene was inserted into exon 2 of *HNF-3α*, producing a gene fusion that allowed us to follow gene expression from the *HNF-3α* promoter in embryoid bodies (EBs) and embryos (Fig. 1). Three independent *HNF-3α* −/− lines were then produced by culturing the *HNF-3α* +/– ES cells in high concentrations of G418 medium, as described (*11*).

Steady-state mRNA concentrations of numerous genes expressed in the VE were measured in 21-day-old *HNF-3α* +/+, +/–, and –/– EBs by reverse transcription polymerase chain reaction (RT-PCR) (Fig. 2). Hypoxanthine phosphoribosyltransferase (HPRT) primers were used to show that each sample contained equivalent amounts of mRNA. Furthermore, mRNA concentrations of VE markers GATA-4 and HNF-1β (*12*) were similar, demonstrating that each sample contained equal amounts of VE. As expected, no HNF-3α product was amplified from *HNF-3α* –/– EBs. Surprisingly, mRNAs for apolipoproteins AI, AII, AIV, B, and CII; albumin; glucose transporter 2; and the glycolytic enzymes aldolase B and L-pyruvate kinase, which are putative targets for HNF-3α, were upregulated three- to eightfold in *HNF-3α* –/– EBs. In addition, mRNA concentrations of HNF-4α and HNF-1α were increased, whereas HNF-3β and -3γ remained relatively unaffected. *LacZ* expression was similar in *HNF-3α* +/– and *HNF-3α* –/– EBs, ruling out any significant transcriptional autoregulation by HNF-3α (*13*). These results demonstrate that, in a native chromosomal context, HNF-3α functions as a repressor of gene expression, including the expression of transcription factors HNF-4α and HNF-1α.

HNF-3α and -3β proteins bind to common DNA recognition sequences as monomers through a conserved DNA binding domain known as the winged helix motif (*14*). *HNF-3α* and *HNF-3β* are sequentially expressed during development in the definitive endoderm, notochord, and floorplate of the neural tube with *HNF-3β* being expressed first (*15*). Targeted disruption of *HNF-3β* produces embryos that lack a notochord and exhibit defects in foregut and neural tube development (*8*). Because *HNF-3α* and *HNF-3β* are coexpressed in these tissues and display similar DNA binding properties, it appeared possible that HNF-3α and HNF-3β have similar functions during early development. To assess whether loss of *HNF-3α* would lead to developmental defects similar to those that have been described for *HNF-3β* null embryos, we generated embryonic day 8.5 (E8.5) *HNF-3α* –/– embryos by tetraploid aggregation and followed the development of notochord, floorplate of the neural tube, and gut by staining for *LacZ* expression (*16*). As shown in Fig. 1B, in contrast to *HNF-3β* −/− embryos (*17*), *HNF-3α* –/– embryos were developmentally indistinguishable from wild-type embryos.

The distinct phenotypes shown by *HNF-3α* –/– and *-3β* –/– embryos suggested that HNF-3α and HNF-3β regulated gene expression by different mechanisms. This led us to investigate target gene expression in *HNF-3β* –/– EBs. *HNF-3β* null ES cells were generated by two targeting strategies (*18*). Expression of *HNF-4α* was markedly reduced in *HNF-3β* −/−, as were several genes known to be in vivo targets of *HNF-4α* (Fig. 3). These genes included apolipoproteins AI, AII, AIV, B, CII, and CIII; transthyretin; aldolase B; and L–pyruvate kinase. Expression of *HNF-1α* was also reduced in the absence of *HNF-3β*. Strikingly, *HNF-3α* mRNA was undetectable in *HNF-3β* null EBs, implying that HNF-3β is absolutely required for *HNF-3α* expression

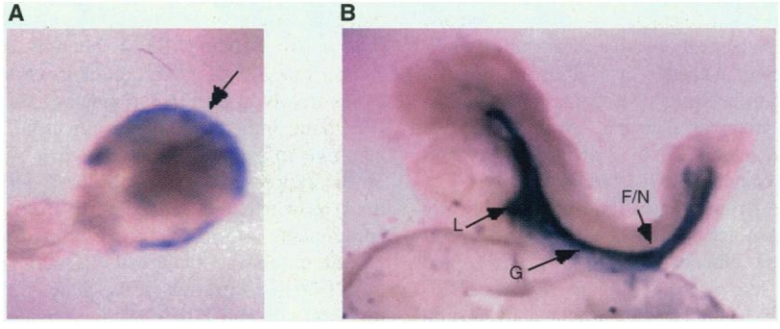

Fig. 1. (A) Expression of *LacZ* in *HNF-3α* –/– ES cell EBs. *HNF-3α* –/– EBs were cultured for 14 days in suspension and then stained for β-galactosidase activity. *LacZ* expression is restricted to the VE (arrow). **(B)** *HNF-3α* –/– ES cell–derived embryos were produced by tetraploid aggregation as described in (*16*). Embryos were harvested at E8.5 and stained for expression of *lacZ*. All embryos were indistinguishable from wild types and exhibited β-galactosidase activity in the floorplate of the neural tube and notochord (F/N) as well as gut (G) and developing liver (L).

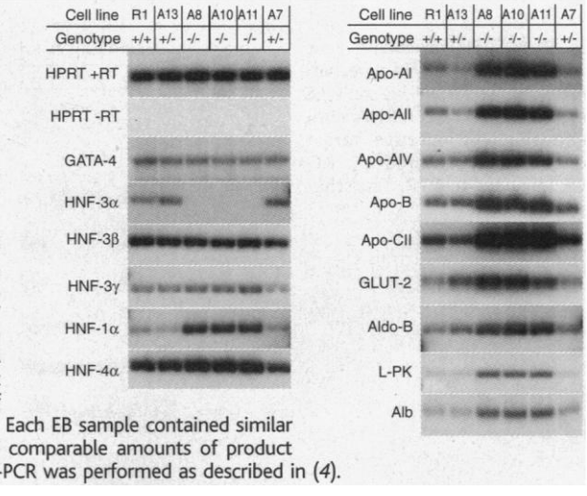

Fig. 2. *HNF-3α*–dependent gene expression in EBs of wild-type, heterozygous, and *HNF-3α* null ES cells. *HNF-3α* +/+ (R1; lane 1), *HNF-3α* +/– (A13 and A7; lanes 2 and 6), and *HNF-3α* –/– (A8, A10, and A11; lanes 3 to 5) were assayed for expression of mRNAs from HPRT; GATA-4; HNF-1α, -3α, -3β, -3γ, and -4α; albumin (Alb); apolipoproteins (APo) AI, AII, AIV, B, and CII; aldolase B (Aldo-B), and L-pyruvate kinase (L-PK). HPRT primers were used to show that each sample started with comparable amounts of cDNA (HPRT +RT) and that no product was amplified in the absence of reverse transcriptase (HPRT –RT). Each EB sample contained similar amounts of VE as shown by the comparable amounts of product amplified by GATA-4 primers. RT-PCR was performed as described in (*4*).

in EBs. Steady-state levels of *HNF-3γ* mRNA were not affected.

Our genetic analyses showed that whereas HNF-3α acted as a negative regulator, HNF-3β was a strong activator of the same target genes. This could suggest that HNF-3α acts as a transcriptional repressor of gene expression in a native chromosomal context. Alternatively, because HNF-3α and HNF-3β interact with identical consensus binding sites as monomers, the possibility was raised that HNF-3α could interfere with the ability of HNF-3β to transactivate by competing for HNF-3 binding sites. To distinguish between these models we expressed HNF-3α in *HNF-3β* –/– EBs and asked whether HNF-3α could restore expression of target genes; if HNF-3α acted as a direct repressor, it should be incapable of achieving this. EBs were generated from wild-type, *HNF-3β* –/–, and three independent *HNF-3β* –/– ES cell lines that expressed a rat *HNF-3α* DNA from the immediate early cytomegalovirus (CMV) promoter (Fig. 4; α3, α4, and α5). The mRNA levels of aldolase B and apolipoprotein CII were restored in *HNF-3β* –/– EBs that expressed *HNF-3α* (Fig. 4). Furthermore, this expression depended on the amount of HNF-3α. From this we conclude that both HNF-3α and HNF-3β are activators of gene expression and that in the context of native chromatin HNF-3α is a poorer activator than HNF-3β. Because both of these factors recognize the same cis-acting promoter elements, we propose that HNF-3α inhibits HNF-3β activity by competing for common HNF-3 binding sites.

The strong regulation of metabolically controlled genes such as enzymes of glycolysis and gluconeogenesis by HNF-3 and HNF-4 led us to investigate whether HNF-3α/HNF-3β ratios were affected by insulin (*19*). We measured steady-state concentrations of *HNF-3α* and *HNF-3β* mRNAs in day14 EBs that were cultured for 24 hours in serum-free medium containing 0, 5, or 50 nM insulin and 20 mM glucose. In the presence of insulin, *HNF-3β* expression was upregulated, whereas *HNF–3α* was reduced (Fig. 5). Moreover, *HNF-4α* expression and downstream targets, such as aldolase B and L–pyruvate kinase, were also significantly increased. We conclude that insulin can act as a positive modulator of the HNF transcription factor network.

Placement of HNF-3β at the top of the transcription factor hierarchy regulating *HNF-4α* and *HNF-3α* is consistent with it being expressed at the earliest stages of fetal development before expression of both *HNF-3α* and *HNF-4α* (*15*). Moreover, whereas *HNF-3β* –/– embryos exhibit severe defects early in embryogenesis, *HNF-3α* –/– embryos are normal at the same developmental stage. Further evidence supporting regulation of *HNF-3α* by HNF-3β comes from analyses of the HNF-3α promoter, which was found to contain an HNF-3 binding site (*20*). However, unlike the VE, the definitive endoderm expresses *HNF-3α* in *HNF-3β* –/– embryos, suggesting that other transcription factors can compensate for loss of *HNF-3β* in this tissue (*8*).

Mutations in the genes encoding HNF-4α and HNF-1α have been identified in families with an early-onset form of type 2 diabetes (MODY1 and -3, respectively), which is characterized by autosomal-dominant inheritance and defects in insulin secretion (*6*). It is likely that the MODY phenotype results from a defect in the expression of genes involved in glucose metabolism (*4*). Our data indicate that the positive and negative impact of HNF-3β and HNF-3α on the expression of *HNF-4α/HNF-1α* and their downstream targets are important components of this complex hierarchical circuit regulating pancreatic β cell function. Genetic variation in the genes encoding HNF-3α and HNF-3β may be responsible for other forms of MODY. Moreover, our findings that this pathway is also regulated by insulin implies that this cascade is also important for insulin action. Interestingly, the insulin receptor–like *DAF-2* gene in *Caenorhabditis elegans* has recently been shown to be an upstream regulator of the HNF-3/forkhead homologue DAF-16 in metabolic control of the dauer larval stage, which suggests that this pathway may be evolutionarily conserved (*21*). Because resistance to insulin is a common fea-

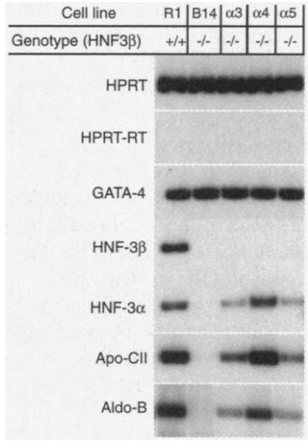

Fig. 4. Expression of *HNF-3α* in *HNF-3β* –/– EBs. A transgene expressing the rat *HNF-3α* cDNA under the control of CMV promoter and containing the hygromycin resistance gene (*hyg*^{R}) was transfected into *HNF-3β* –/– ES cell line B14. Transfectants were grown in the presence of hygromycin (0.4 mg/ml) for 8 days and three resistant lines were used to generate EBs. Steady-state mRNA concentrations were measured by RT-PCR in 14-day-old EBs. Concentrations of *HNF-3α* mRNA were moderate in lines α3 and α5 and highest in line α4. The rescue of *HNF-3α* expression in *HNF-3β* –/– EBs led to dose-dependent transcriptional activation of the target genes *apoCII* and *aldo-B*.

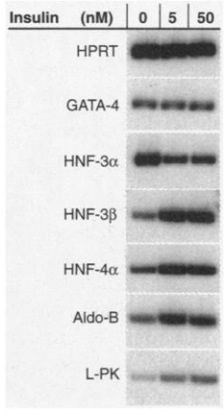

Fig. 5. Insulin regulates expression of *HNF-3α*, *HNF-3β*, and downstream target genes. Day 14 EBs were grown in 2.5% fetal calf serum for 12 hours followed by serum-free Dulbecco's minimum essential medium overnight. EBs were then cultured in serum-free medium containing 20 mM glucose and 0, 5, or 50 mM insulin for 24 hours. Steady-state mRNA concentrations of HPRT, GATA-4, HNF-3α, HNF-3β, HNF-4α, Aldo-B, and L-PK mRNA were determined by RT-PCR.

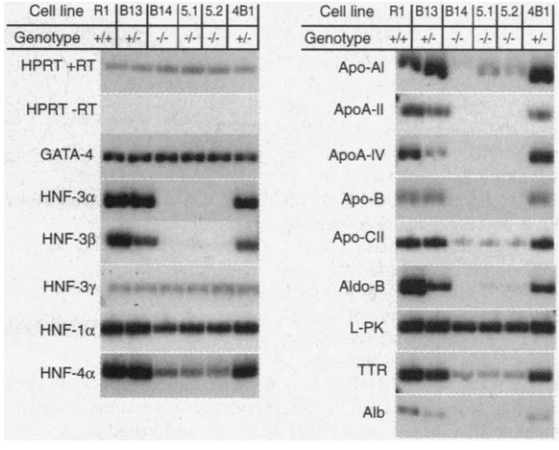

Fig. 3. *HNF-3β*–dependent gene expression in EBs of wild-type, heterozygous, and *HNF-3β* null ES cells. Steady-state mRNA concentrations of putative target genes were measured by RT-PCR (Fig. 2) (*4*). TTR, Transthyretin.

ture of late-onset non-insulin-dependent diabetes mellitus, it is possible that dysregulation of the HNF regulatory pathway, whether primary or secondary, can also contribute to this complex metabolic syndrome.

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10. The HNF-3α targeting vector was constructed by deleting the DNA binding and transactivation domains between the Afl III and Nhe I sites in exon 2. The *E. coli lacZ* gene was fused in frame to exon 2 of *HNF-3α*, which placed it under the control of *HNF-3α* transcriptional regulatory sequences. Recombinant ES cell clones were genotyped by Southern blotting. DNAs were digested with Hind III–Eco RV and blots were probed with *HNF-3α* genomic DNA, which lay 5′ to sequences present in the targeting vector. The wild-type allele produced a 7.5-kb restriction fragment, whereas the targeted allele produced a 4.5-kb fragment. *HNF-3α −/−* ES cells were selected by growing HNF-3α in medium containing G418 (2.0 mg/ml). Three independent *HNF-3α −/−* (A8, A10, A11) lines were cloned and further analyzed.
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17. *HNF-3β −/−* embryos were generated by crossing *HNF-3β +/−* mice. Embryos were dissected from the uterus at day E8.5 and stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside. Results obtained (data not shown) were as described in (*8*).
18. The production of *HNF-3β +/−* ES cell lines, B13 and 4B1, has been described (*8*). Genotypes of *HNF-3β* mutant ES cell lines were analyzed by Southern blotting (*8*). *HNF-3β −/−* line B14 was derived from *HNF-3β +/−* line B13, and *HNF-3β −/−* lines 5.2 and 5.1 are from 4B1 as described in (*8*).
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Impact of a Catastrophic Hurricane on Island Populations

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Lizard and spider populations were censused immediately before and after Hurricane Lili on islands differentially affected by the storm surge. The results support three general propositions. First, the larger organisms, lizards, are more resistant to the immediate impact of moderate disturbance, whereas the more prolific spiders recover faster. Second, extinction risk is related to population size when disturbance is moderate but not when it is catastrophic. Third, after catastrophic disturbance, the recovery rate among different types of organisms is related to dispersal ability. The absence of the poorer dispersers, lizards, from many suitable islands is probably the result of long-lasting effects of catastrophes.

Major natural disturbances such as hurricanes and volcanic eruptions may be so catastrophic that biotas in exposed areas are scarred and even reshaped for years to come. Although major disturbances are potentially of such great importance (*1*), few precise field data are available to evaluate their impact. Reliable information on the biota before the disturbance is often absent, as is the case for the most lengthily monitored major disturbance of all, the eruption of Krakatoa's volcano in 1883 (*2*). Furthermore, because major disturbances are rare, the initial impact and the incipient stage of recovery are often missed because of the low probability of scientists being in just the right place at the right time.

In October of 1996, the highly improbable happened. We had just finished a census of lizard and spider populations on 19 islands (Fig. 1) near Great Exuma, Bahamas, as part of a long-term experimental study of the ecological effects of introducing two lizard species (*Anolis sagrei* and *A. carolinensis*) (*3*, *4*). Vegetation profiles of each island, which change little under normal conditions, had been determined 2 years previously (*5*). During the early morning of 19 October, Hurricane Lili, the first major hurricane to strike anywhere in the Exumas since 1932 (*6*), passed directly over our study site (Fig. 1) with sustained winds of 90 knots and a storm surge of nearly 5 m (*7*). The study islands are located on both sides of the very large island of Great Exuma. Because Lili's approach was westerly, the 11 islands on the southwest side were exposed to the full force of the storm surge, whereas the 8 islands on the northeast side were protected from this aspect of the hurricane. The next day, as soon as the storm subsided, and for 3 days thereafter, we recensused populations on all the islands. All populations were again censused about 1 year later (23 to 28 September 1997).

These unique data allow us to evaluate several propositions concerning the impact of disturbances on different types of organisms. First, that larger organisms may be more resistant than smaller ones to the immediate impact of a moderate disturbance (*8*, *9*). Second, that surviving smaller species may recover faster because their reproductive rate is higher than that of larger species (*9*). Third, that for moderate disturbances, the risk of extinction is a function of population size, whereas no such relationship exists for catastrophic disturbances (*10*). Fourth, that when all populations are exterminated by a catastrophic disturbance, the recovery rates of different species will be largely determined by their dispersal abilities (*11*). To evaluate

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