studies measuring the amounts of apo A-1 and apo A-2 present in bile in subjects without gallstones but whose bile is supersaturated with cholesterol, particularly in relation to apo A-1 and apo A-2 serum concentrations from these patients, are needed. Also, isolation, purification, and characterization of biliary apo A-1 and apo A-2 from these same patients is needed to understand better the roles of the apolipoproteins. Evidence for the presence of nucleating factors in the gallbladder bile of animal models or patients with cholesterol gallstones has been shown (*13*). Thus, formation of cholesterol gallstones may represent a dynamic process reflecting a balance between nucleating and antinucleating factors in the milieu of bile supersaturated in cholesterol. Whenever an isolated protein is recombined with lipid in vitro, it may not be as effective as would be the case in its normal in vivo state. Nevertheless, the magnitude of the prolongation of nucleation time (that is, only a twofold increase) observed in vitro in our study may not be sufficient to prevent gallstone formation in all susceptible patients. Finally, we speculate that a quantitative or qualitative (that is, functional) deficiency in the effector proteins capable of inhibition of nucleation, such as apo A-1 and apo A-2, may constitute a second important risk factor for cholesterol cholelithiasis in addition to supersaturation of bile in cholesterol.

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8. Nucleation times were determined by polarizing microscopy as described (*3*) with several modifications. Each heated isotropic mixture was filtered through a detergent-free, 0.22-μm filter (Millipore) into a 2-ml capped glass micro-vial; the vial was pressurized with N_2, sealed, and incubated at 37°C. The interval between time zero and the first detection of birefringent cholesterol monohydrate crystals was designated the nucleation time. As is well known, in the microparticulate size range of these experiments a distinction between crystal growth from nuclei versus true nucleation is difficult to determine, and these two processes have often been thought to occur simultaneously. A model bile was constructed by a combination of methods (*1, 2*). The cholesterol saturation index was calculated from the total and relative biliary lipid compositions [M. C. Carey, *J. Lipid Res.* **19**, 945 (1978)].
9. The model bile solutions were incubated at 55°C with shaking until they were isotropic. The solutions were recombined with the protein solutions in Hepes buffer and saline (0.01*M* and 0.14*M*, respectively) at *p*H 7.5 and heated at 37°C; controls were recombined with an identical amount of Hepes buffer–saline only. Both solutions were incubated at 45°C for 5 hours. High-density $d = 1.063$ to 1.21 g/ml) and low-density $d < 1.006$ g/ml) lipoproteins were obtained from normal, pooled human plasma by sequential ultracentrifugation [R. J. Havel, *J. Clin. Invest.* **34**, 1345 (1955)]. Apolipoproteins A-1, A-2, and C-3 were purified as described [S. J. T. Mao *et al.*, *Biochemistry* **14**, 4127 (1975); S. I. Barr *et al.*, *Biochim. Biophys. Acta* **663**, 491 (1981)].
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11. Proteins were measured fluorometrically by a modified fluorescamine assay [J. V. Castell, M. Cervera, R. Marco, *Anal. Biochem.* **99**, 379 (1979)] after trichloroacetic acid precipitation, organic delipidation, and alkaline hydrolysis (*5*). Cholesterol, phospholipid, and bile salts were measured as described (*3*).
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Nucleotide Sequence of a Human *Blym* Transforming Gene Activated in a Burkitt's Lymphoma

Abstract. *The nucleotide sequence of a human* Blym-*1 transforming gene activated in a Burkitt's lymphoma cell line was determined. This sequence predicts a small protein of 58 amino acids that is 33 percent identical to the predicted product of chicken* Blym-*1, the activated transforming gene of chicken B cell lymphomas. Both the human and chicken* Blym-*1 genes exhibit significant identity to an amino-terminal region of transferrins.*

The *Blym*-1 transforming gene was first isolated by transfection of NIH 3T3 cells with DNA of chicken B cell lymphomas (*1, 2*). The nucleotide sequence of chicken *Blym*-1 indicated that it encoded a small protein of 65 amino acids that was partially identical to the amino-terminal region of transferrin family proteins (*2*). The cloned chicken *Blym*-1 gene hybridized to a small family of sequences in DNA's of both chicken and human cells, suggesting that it was a member of a small gene family that was conserved in vertebrate evolution (*2*). Subsequently, a biologically active transforming gene detected by transfection of DNA's of six Burkitt's lymphoma cell lines was isolated with the use of chicken *Blym*-1 as a hybridization probe, indicating that the transforming gene activated in these human B cell neoplasms was a member of the gene family defined by hybridization to chicken *Blym*-1 (*3*). We now present the complete nucleotide sequence of the transforming gene (designated human *Blym*-1) isolated from one of these Burkitt's lymphoma cell lines and describe its relationship to chicken *Blym*-1 and to transferrin.

Earlier we showed that the entire biologically active human *Blym*-1 gene was contained within a 1.0-kilobase (kb) Eco RI fragment (*3*). This fragment was therefore subcloned into the Eco RI site of pBR322 for sequence analysis. The resulting plasmid, pHuBlym-1, induced transformation of NIH 3T3 cells with an efficiency of approximately 4.5×10^3 foci per microgram of DNA. For sequencing by the method of Maxam and Gilbert (*4*) (Fig. 1), pHuBlym-1 was digested with Eco RI, Pst I, or Ava I, labeled at either the 5′ or 3′ end with ^{32}P, digested with an appropriate second restriction enzyme, and purified by gel electrophoresis. For sequencing by the dideoxy chain-termination procedure (*5*) (Fig. 1), the 1.0-kb Eco RI fragment was isolated and digested with Pst I, and the resulting Eco RI–Pst I fragments were inserted in both possible orientations into the M13 bacteriophage vectors mp8 and mp9 (*6*). Most of the sequence was determined from both strands. In addition, all of the sequence except for 33 nucleotides (positions 265 to 297) was determined from two independent clones.

The nucleotide sequence of the 1.0-kb Eco RI fragment containing human *Blym*-1 (Fig. 1) confirms the positions of the Pst I and Ava I restriction enzyme sites, which we had previously mapped within this region. However the nucleotide sequence did not identify a Bam HI site, which was mapped in the initial λ clone of human *Blym*-1 approximately 200 nucleotides from the left-hand

Eco RI site (*3*). Consistent with this result, digestion of pHuBlym-1 with Bam HI revealed that the cleavage site previously mapped in the insert was digested less efficiently than the Bam HI site in plasmid DNA. Complete digestion of the Bam HI site in the insert required an excess of enzyme, similar to the conditions previously employed for digestion of the λ clone. These results indicate that the Bam HI site mapped within the human *Blym*-1 gene represents a sequence that is not a complete Bam HI recognition site but is cleaved by Bam HI under conditions of enzyme excess. One possibility for such a site is the sequence GGATCAC at position 460 (G, guanine; A, adenine; T, thymine; C, cytosine), which is similar but not identical to the canonical Bam HI recognition site (GGATCC).

The sequence presented in Fig. 1 was analyzed for signals characteristic of eukaryotic transcription initiation and termination to identify the human *Blym*-1 transcription unit. A single polyadenylation sequence (AATAAA) (*7*) is present at position 700, indicating the 3′ terminus of the human *Blym*-1 messenger RNA (mRNA). 5′ of the polyadenylation signal, sequences associated with eukaryotic promoters (*8*) can be identified: the sequence CCAT which occurs both at positions 8 and 20, and the sequence AATATTTATA at position 63. If these sequences define the promoter of human *Blym*-1, transcription would be expected to initiate approximately 25 nucleotides downstream from the TATA box, around position 90. The first ATG translation start codon would then be at position 107. This reading frame is interrupted at position 164 by the TAA stop codon, suggesting that the consensus splice donor sequence (*9*) AG/GT at positions 155 to 158 serves as a probable 5′-splice site. A consensus splice acceptor sequence (*9*), CAG/, which is present in a large open reading frame at positions 532 to 534, could then serve as the 3′-splice recipient site. If these signals are utilized, translation of a mature, spliced mRNA would begin at the ATG sequence at position 107 and terminate at the TGA stop codon at position 659. Northern blot analysis with a human *Blym*-1 probe has identified a polyadenylated RNA of approximately 500 nucleotides in Burkitt's lymphoma cell lines (data not shown), consistent with the size of the polyadenylated mRNA predicted by the sequence. The predicted translation product of this mRNA is a small protein of 58 amino acids (Fig. 1).

Blot hybridization experiments indicated that pHuBlym-1 hybridized to repeated elements present in the human genome, although a unique cellular sequence could be detected under highly stringent hybridization conditions (*3*). We therefore scanned the human *Blym*-1 sequence for known repetitive elements. This revealed a region of 51 nucleotides within the intron of human *Blym*-1 (positions 266 to 313), which showed 88 percent identity to the consensus sequence of the Alu family of moderately repeated sequences (*10*).

The predicted amino acid sequence of the *Blym*-1 gene product (58 amino acids) is similar in size to that of chicken *Blym*-1 (65 amino acids). In addition, both genes are 600 to 700 nucleotides in length and are interrupted by a single intervening sequence separating a small first exon from a larger second exon. The deduced amino acid sequence of human *Blym*-1, like that of chicken *Blym*-1, is rich in lysine and arginine (21 percent for both proteins). When the predicted ami-

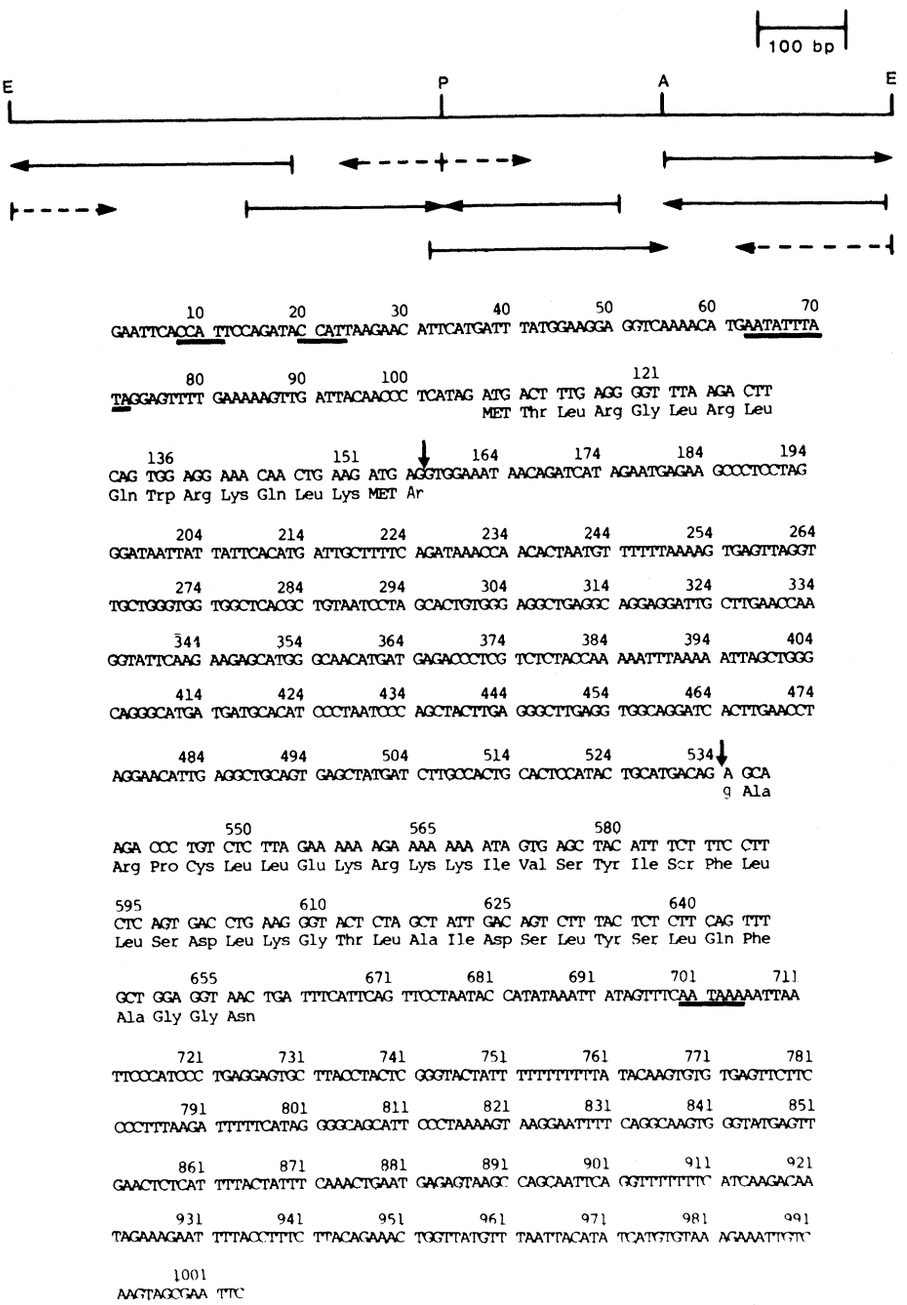


Fig. 1. Human *Blym*-1 nucleotide sequence. (Top) Restriction map and sequencing strategy of the cellular insert of pHuBlym-1 showing the positions of cleavage sites for the restriction enzymes Eco RI (E), Pst I (P), and Ava I (A). Beneath the map, the extent of sequencing and the polarity of the strand sequenced are indicated by arrows. Solid arrows indicate sequencing by the Maxam and Gilbert method (*4*), and dashed arrows indicate sequencing by the dideoxy chain-termination method (*5*). (Bottom) The nucleotide sequence with the predicted amino acid sequence below it. Sequences associated with promoters and polyadenylation signals are underlined. Arrows indicate the positions of putative splice sites.

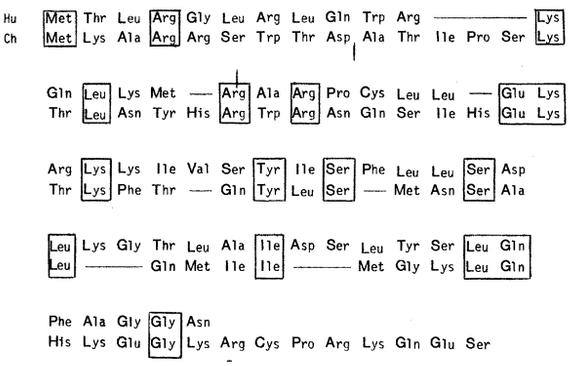


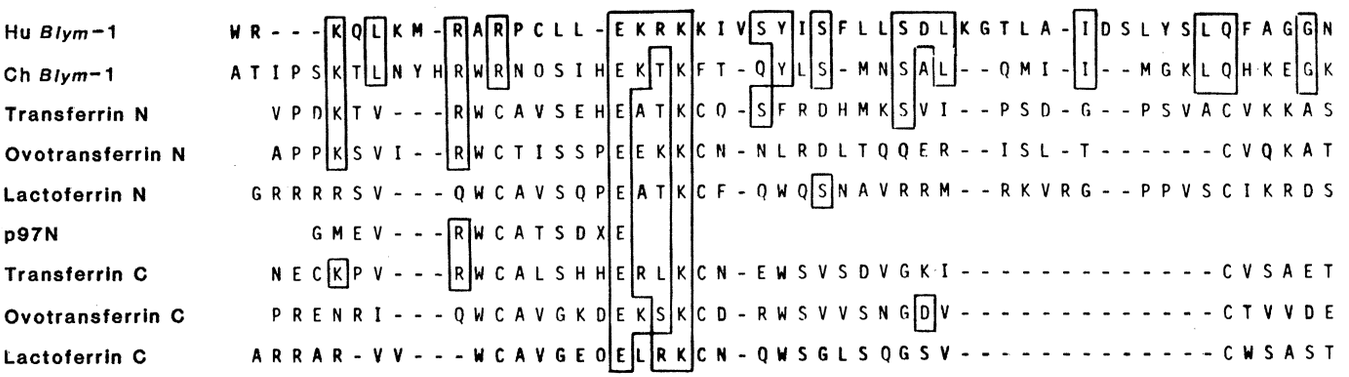
Fig. 2. Comparison of the translated amino acid sequences of human (Hu) and chicken (Ch) *Blym*-1. Amino acids common to both sequences are boxed. Dashes indicate gaps introduced to maximize the alignment. Vertical lines indicate the position of the splice sites.

no acid sequences of both proteins were aligned (Fig. 2), 17 identities were observed among 52 aligned amino acids (33 percent overall identity). The two proteins are related ($P < 0.005$) (*11*), although significant divergence between the sequences has occurred.

The chicken and human *Blym*-1 genes exhibited 50 to 60 percent nucleotide sequence identity overall, with several short regions of 15 to 25 nucleotides exhibiting 70 to 75 percent identity. This degree of nucleotide similarity is consistent with the observed amino acid sequence relationship and with the previous observation that cross hybridization of the two genes was weak and required low stringency conditions for detection (*3*).

Chicken *Blym*-1 does hybridize more strongly to other sequences in human DNA (*2*). If these more strongly hybridizing sequences represent functional genes, human *Blym*-1 would be implicated as a member of the *Blym* family that is relatively divergent from chicken *Blym*-1.

The chicken *Blym*-1 gene product was reported as showing a significant relationship to the amino-terminal region of the transferrin family of proteins (*2*). Transferrins have evolved by gene duplication, resulting in related amino- and carboxyl-terminal halves (*12*). The amino acid sequence of the second exon of chicken *Blym*-1 is related to an amino-terminal region within each half (*2*). This feature is also shared by human *Blym*-1. The equivalent amino acid sequence of



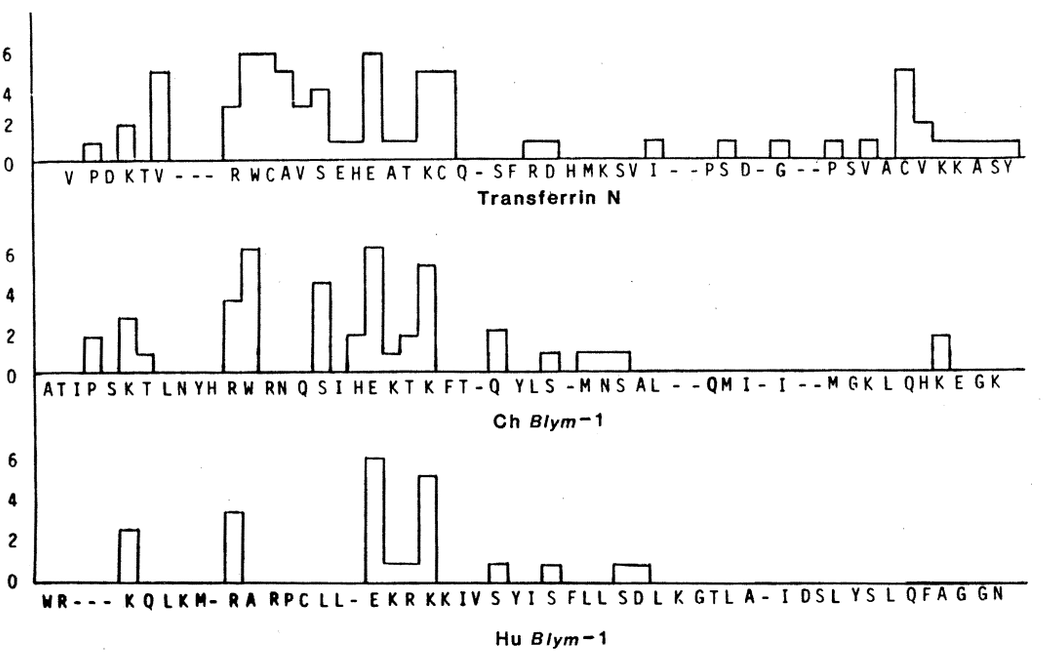


Fig. 3. Homology of the human and chicken *Blym*-1 proteins to the amino-terminal region of transferrins. (Top) Amino acids common to human *Blym*-1 and either chicken *Blym*-1 or at least one member of the transferrin family are enclosed in boxes. Included in this comparison are the related amino-terminal regions of both the amino (N)- and carboxyl (C)-terminal halves of human transferrin, chicken ovotransferrin, lactoferrin (*12*), and the amino-terminal region of p97 (*13*). (Bottom) The predicted amino acid sequences of human *Blym*-1, chicken *Blym*-1, and human transferrin (N) are individually compared to the corresponding regions of the amino-terminal halves of ovotransferrin, lactoferrin, and p97 and the carboxyl-terminal halves of human transferrin, ovotransferrin, and lactoferrin. Below the horizontal axis are the amino acid sequences of human *Blym*-1, chicken *Blym*-1, and human transferrin. Above each amino acid, the height of the graph indicates how many of the six transferrin family sequences have the same amino acid at that position. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

human *Blym*-1 was compared to the amino-terminal regions of both the amino- and carboxyl-terminal halves of human transferrin, ovotransferrin, and lactoferrin (*12*) and to the amino-terminal region of p97, a melanoma surface antigen that is also a member of the transferrin family (*13*) (Fig. 3). The alignment of chicken *Blym*-1 with these transferrin sequences was the same as that previously reported (*2*). The alignment of human *Blym*-1 was fixed by its alignment with chicken *Blym*-1 (Fig. 2). Human *Blym*-1, like chicken *Blym*-1, is related to these amino-terminal transferrin sequences. Significantly, those residues that are conserved between the human and chicken *Blym*-1 genes tend to be conserved among the transferrins as well. If the amino acid sequence of human *Blym*-1 is compared to the amino-terminal region of human transferrin, there are six identities of 39 aligned amino acids ($P < 0.005$) (*11*). Of these six residues, five are also conserved in chicken *Blym*-1. This analysis can be extended to include the other transferrin family sequences. This reveals ten residues of human *Blym*-1 that are conserved in at least one of the transferrin sequences. Seven of these ten amino acids are also conserved between human and chicken *Blym*-1.

Human transferrin and both *Blym*-1 genes display a common pattern of sequence conservation and divergence, with this pattern being somewhat stronger for chicken than for human *Blym*-1 (Fig. 3). For example, the similarity of both *Blym*-1 genes and of human transferrin to other transferrin family sequences is highly conserved in the 5′ half of the indicated sequence but divergent in the 3′ half. Such divergent genes as chicken and human *Blym*-1 are unlikely to have maintained this relationship to transferrin by chance. Rather, the conserved similarity of both *Blym*-1 gene products to transferrin suggests that this relationship reflects some functional property of the *Blym*-1 transforming proteins.

Transferrins are a family of large, iron-binding proteins that are essential growth factors for cultured cells (*14*). A correlation exists between the appearance of transferrin receptors and cell proliferation, suggesting that transferrins may play a role in cell growth (*15*). In support of this concept, it has been shown that transferrin can serve as a lymphocyte mitogen and that blockage of the transferrin receptor with monoclonal antibodies can inhibit cell proliferation even if iron is supplied by alternate mechanisms (*16*). Furthermore, p21, the product of the *ras* transforming gene, has been shown to form a stable complex with the transferrin receptor, suggesting that p21 may exert its effect on cell proliferation in conjunction with transferrin and its surface receptor (*17*). The observed structural relationship between the *Blym*-1 genes and transferrins thus suggests that the *Blym*-1 transforming gene products may also function via a pathway related to transferrin.

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Variation Among Floral Visitors in Pollination Ability: A Precondition for Mutualism Specialization

Abstract. *The unusual floral biology of a neotropical herb provided an opportunity to determine that floral visitors varied significantly in their ability to effect fruit-set. Pollination efficiency and visitation frequency varied among Hymenoptera (five taxa), which were responsible for 99 percent of all fruits set. Lepidoptera (four taxa) were common visitors but poor pollinators. These results indicate that flower visitors vary in their beneficial effects on plants, fulfilling one of the primary conditions required for the specialization of plants on pollinators.*

Despite their ubiquity, mutualisms are perhaps the most poorly understood of all ecological interactions (*1, 2*). Questions of general interest, for which there is little empirical information, include whether potential mutualists vary in quality and what features promote specialization in mutualisms (*2–4*). We report that the visitors to flowers of *Calathea ovandensis* (Marantaceae), a neotropical herb, vary significantly in visitation frequency and their ability to effect fruit-set, two components of pollination efficiency. These results show that the selective effects of potential pollinators on plants can be highly variable, fulfilling one of the primary conditions required for the evolutionary specialization of mutualisms.

The principle of "the most effective pollinator" is central to discussions of plant-pollinator evolution (*3, 5*), but no other study has used seed set as a measure of pollinator efficiency in addition to examining the full range of floral visitors in a natural community (*6–8*). Such an approach is necessary for two reasons: (i) seed set is a direct measure of reproductive success; and (ii) assessment of the relative contribution of each visitor to plant reproductive success requires sampling the entire visitor fauna. Sampling only the most abundant visitors may be misleading if pollination efficien-