AGE-SPECIFIC REPRODUCTIVE STRATEGIES AND DELAYED EMBRYONIC DEVELOPMENT IN AN OLD WORLD FRUIT BAT, *PTENOCHIRUS JAGORI*

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Ptenochirus jagori (Megachiroptera, Pteropodidae) is a tropical cynopterine fruit bat restricted to the Philippine islands. Samples of bats were obtained over 4 years, and reproductive tracts were preserved and examined using standard histological techniques. Reproductive status also was recorded for bats captured, marked, and released. A facultative post-implantational delay in embryonic development was detected in young *P. jagori* females reproducing for the first time. The delay occurred at the stage of gastrulation and could last up to 5 months. Adult females showed little evidence of a delay in development. With this delay, young females gave birth only once in their first year and synchronized parturitions with those of adults. Adult females had a gestation period of 4 months and gave birth twice a year, once in late March and early April and once in August. Morphology of early development was similar to that of other cynopterine fruit bats. The evolution and significance of a post-implantational delay in development are discussed.

Key words: *Ptenochirus*, reproduction, reproductive timing, embryonic development, delayed development, breeding

Fitness of an individual often depends critically on its reproductive strategy, which may vary dramatically with age. An inexperienced young animal may achieve higher lifetime reproductive success by following a conservative reproductive strategy until it gains experience and then switching to a riskier strategy involving greater reproductive investment and potential for greater production of offspring later in life.

The order Chiroptera includes a remarkably broad range of variation in reproductive strategies, morphology, and physiology, including much of the variation seen across all other orders of mammals and a number of unique specializations (Mossman, 1987; Racey, 1982). Bats time reproduction using periods of reproductive quiescence, sperm storage by both males and females, delayed ovulation and fertilization, delayed implantation, and delayed development (Racey, 1982). Bats make use of a variety of environmental signals and endogenous rhythms to time reproductive events (Beasley, 1985–1986; Heideman and Bronson, 1994; Martin et al., 1995). These strategies allow bats to achieve a high degree of synchrony of births, even in the tropics. Correlational evidence from many studies suggests that natural selection often has favored reproductive timing that synchronizes late pregnancy, lactation, or weaning with peaks in food resources (Dinerstein, 1986; Racey, 1982).

A rare and poorly understood method of regulating reproductive timing is known only in bats—post-implantational delayed development (Bradshaw, 1962; Fleming et al., 1972), in which implanted embryos halt or nearly halt development for periods up to 8 months (Heideman, 1989*a*). The delay occurs at a specific stage of development, generally just before or at gastrulation. The period of delay is similar for all females in some species (Bleier, 1975; Fleming et al., 1972) but can be highly variable in others (Heideman, 1988, 1989*a*). Delays do not require lowered body temperature (Burns et al., 1972), unlike periods of retarded embryonic growth that occur in many species of bats at times when body temperature is reduced (Heideman, in press; Racey, 1982). Delayed development has been described only in a few species of bats in several fam-

ilies (Bradshaw, 1962; Fleming, 1971; Heideman et al., 1993; Rasweiler and Badwaik, 1997), including examples in three genera of cynopterine megachiropterans (Heideman, 1988, 1989*a*; Heideman et al., 1993). In addition, evidence from changes in uterine mass during pregnancy suggests that delayed development may occur in *Cynopterus sphinx* (Krishna and Dominic, 1983). The ability of embryos to halt embryonic development implies existence of a developmental switch whose physiological and molecular mechanisms are unknown.

We report a difference in reproductive strategies between young female *Ptenochirus jagori* (Megachiroptera, Pteropodidae) in their first reproductive attempt and older parous females. Most young females apparently incorporate a facultative developmental delay into their first pregnancy, but there is no (or little) such delay in older females. Use of delayed development may allow greater flexibility of reproductive timing in young females.

Our objectives were to 1) describe a post-implantational delay in development in the embryos of young adult female *P. jagori,* 2) describe timing of early development in this context, 3) compare patterns and timing of reproduction in young and full adult *P. jagori,* and 4) discuss the evolution and significance of a post-implantational delay in development.

Materials and Methods

Data were collected between 1979 and 1984 in the Lake Balinsasayao watershed on Negros Island in the Philippine Archipelago (9°11′N, 121°23′E). Specimens were deposited in the collection of the University of Michigan Museum of Zoology. Additional data were collected from individuals released as part of a population study (Heideman and Heaney, 1989). The study area was mainly primary forest. Mean annual temperature was 22°C, with a mean low of 19°C and mean high of 25°C (Heideman and Erickson, 1987). Total annual rainfall at the site is ca. 3,100 mm; moderate seasonal differences in rainfall resulted in a mild dry season from March to May (Heideman and Heaney, 1989). Flower and fruit production tended to be mildly seasonal, with a peak in flowering in the dry season and early wet season and a small, but statistically non-significant, peak in fruiting at the beginning of the wet season (Heideman, 1989*b*). A more detailed description of the study area and collection methods was presented by Heideman and Heaney (1989).

Ptenochirus jagori is a tropical frugivore with an average weight of 87 g (Heideman and Heaney, 1989) and a diet including figs (*Ficus*) and wild bananas (*Musa*); other fruits, leaves, and flowers are likely food resources (Heideman and Heaney, 1992; Utzurrum, 1984, 1995; Utzurrum and Heideman, 1991). This tropical cynopterine fruit bat is restricted to the Philippine Islands (Heaney et al., 1987) where it is locally abundant (Heideman and Heaney, 1989; Ingle, 1992). On Negros Island, there are two annual birth peaks, one in early March and one in early August (Heideman, 1995); on Luzon, the two birth periods occur in late April/early May and September (Ingle, 1992; Mudar and Allen, 1986). Gestation in adults is ca. 4 months, followed by 2–3 months of lactation (Heideman, 1995).

Specimens described here were collected in June 1981 and monthly from June 1982 to June 1983; others captured in 1984 were examined for reproductive condition and released. Ninety-six of 225 females captured between 1981 and 1983 were selected for reproductive necropsies. For these females, teat size, development of mammary tissue, lactational status, greatest length of conceptus (embryo with surrounding fetal and maternal tissue, including the uterus), ovarian size, and ovarian color variation were recorded. Reproductive tracts were removed, fixed in Bouin's solution, dehydrated in a graded ethanol series, embedded in Paraplast, serially sectioned at 5–10 μm, and stained with hematoxylin and eosin or a modified Crossman-Mallory trichrome stain for light microscopy.

The other females were marked and released after external examination to determine reproductive status, including palpation for embryos. Bats were assigned to age classes on the basis of wing-joint development and teat size. Full adults, recognized by fused phalangeal epiphyses and "knobby" joints, had medium or large teats (Barbour and Davis, 1969; Heideman, 1988). Young adults were defined as females that had not yet completed growth of the epiphyses or still had small teats; pregnant females

with these characteristics were assumed to be reproducing for the first time. Only females ≥68 g were included in analyses and illustrated in figures because although many females that weighed 68 g were pregnant, none weighing <68 g were pregnant. Subadults were defined as females that weighed <68 g, had unfused phalangeal epiphyses and joints that appeared swollen in comparison to adults, had tiny teats, and were not pregnant.

Accuracy rates for palpation were determined using data from necropsied bats. Palpation for embryos was conducted before reproductive necropsy, and results were used to assess the error rate for detection of embryos and for the estimation of size of embryos. Ninety-six percent of females were categorized correctly as palpably pregnant or not palpably pregnant (where palpably pregnant refers to presence of embryos >2 mm). Non-pregnant females and females with a conceptus ≤5 mm in greatest length were most likely to be assessed incorrectly. In this group, 94% were categorized correctly, but 98% of females carrying a conceptus >5 mm were identified correctly as pregnant. Unimplanted embryos were undectable by palpation, and some early implanting embryos also were undetectable by palpation. Estimates of conceptus size by palpation were generally within 1–3 mm of actual embryo sizes; 84% of estimates from palpation were within 20% of the actual size. It is worth noting that skill and accuracy of assessment of pregnancy by palpation of small embryos were facilitated by the immediate feedback provided by necropsy and measurement of embryos immediately after palpation of some females of *P. jagori* and other species. All P values are from binomial tests (Sokal and Rohlf, 1981). Differences were considered significant if $P < 0.05$.

Results

Reproductive morphology.—The single pair of pectoral mammae have teats that were large (ca. 5 mm in length and 5 mm in width) and conspicuous on females that had nursed previously; teats were small (ca. 1 mm in length and width) and flat against the body on most young adult and all subadult females. Teats of young females were small until the first pregnancy, during which the teats grew, and by mid-pregnancy, teats of some young females were similar in size to those of adults. Teats become larger still during the first period of suckling and apparently remained large thereafter.

Ovaries were 1–2 mm in diameter. Presence of corpora lutea was sometimes detectable macroscopically, and in some cases, a 2-mm ovary swollen with a corpus luteum could be detected by palpation as a second swelling just cranial to the conceptus. The ovarian bursa enclosed the ovary completely except for a small slit-shaped opening at the point where the infundibulum contacts or nearly contacts the uterus (Figs. 1a, 1b, and 1c). The oviduct curved cranially from the infundibulum over the ovary and then projected caudally to meet and open into the cranial tip of the uterus.

Ptenochirus jagori has a long duplex uterus. Quiescent uteri were 4–9 mm in length and 1 mm in diameter. The uteri join superficially at the cervix. In formalin-preserved specimens, the vulva was partially covered by a thick fold of skin, but this was not observed in live females.

In cross-section, the quiescent uterus had a small lumen and moderate development of uterine glands. *P. jagori* exhibited a slight uterine progestational reaction. For both young and full adults with tubal zygotes or pre-implantation embryos, the cranial one-fourth of the ipsilateral uterine horn was larger in diameter by 29% (n = 5) than the corresponding contralateral horn. In contrast, size differences between the two uterine horns of nonpregnant bats were small (range 0–7%). In the area of the progestational reaction, uterine glands were longer and uterine tissue had hypertrophied to the point that the uterine lumen was compressed closed. For young adults and adults with implanting embryos, the increase in diameter of the progestational horn ranged from 1–33% (n = 5) due to the increased development of uterine glands relative to the contralateral horn. In females with tubal zygotes and in some females with implanting embryos, epithelial cells of the proges-

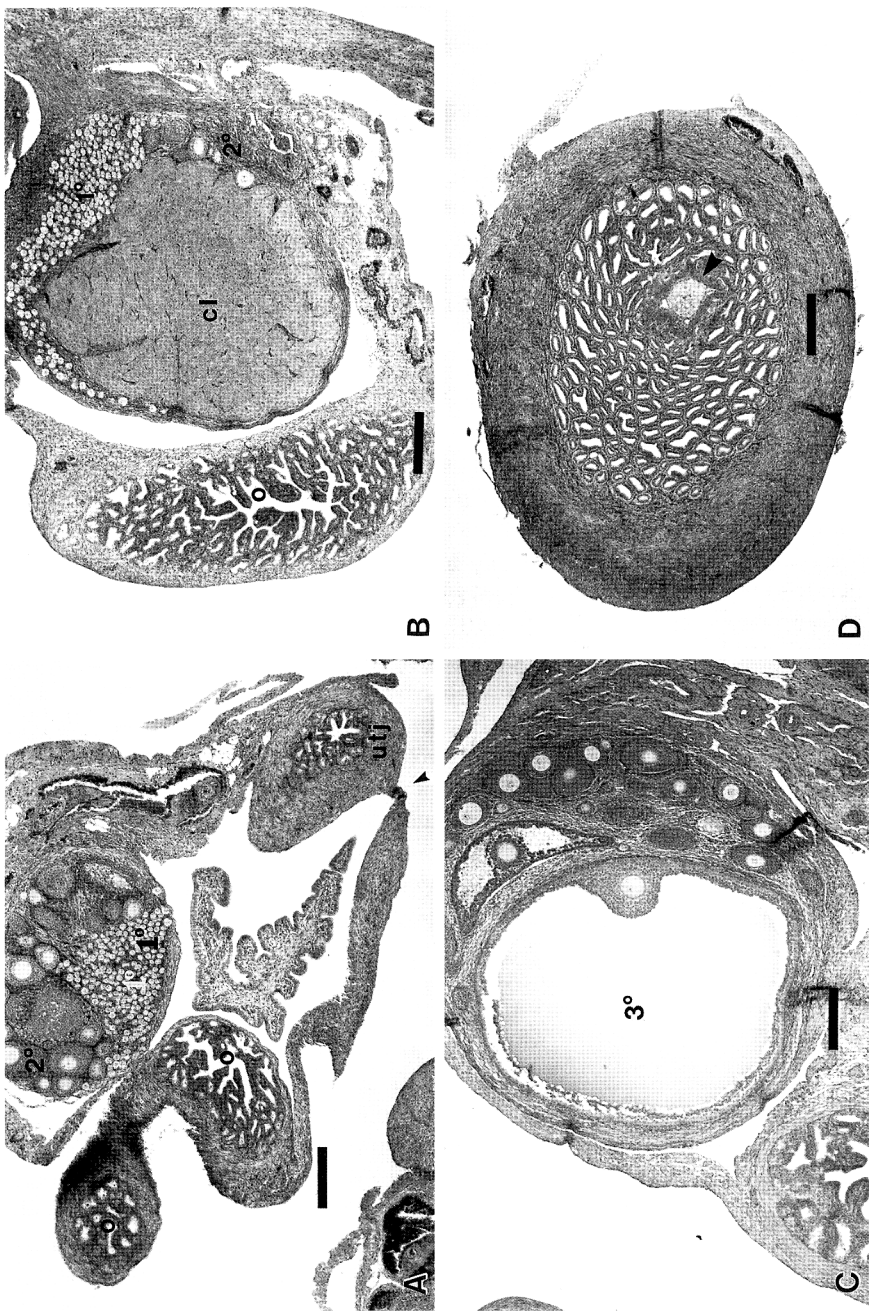

FIG. 1.—A) Oviduct (o) and contralateral ovary from a young adult with an early implanting blastocyst on 9 December 1982 containing primary (1°) and secondary (2°) follicles. Arrowhead indicates location of slit in ovarian bursa about 100 μm caudal to this section. utj: uterotubal junction. B) Oviduct and ipsilateral ovary with corpus luteum (cl) from a young adult with an early implanting blastocyst on 9 December 1982. C) Ovary from an adult with a large tertiary follicle (3°) near ovulation on 5 November 1982. Primary and secondary follicles are visible to the right of the large follicle. D) Early implanting blastocyst (arrowhead) centered within the progestational region of the uterus of a young adult captured on 9 December 1982. Ovaries of this individual are shown in Figures 1a and 1b. Scale bars = 200 μm.

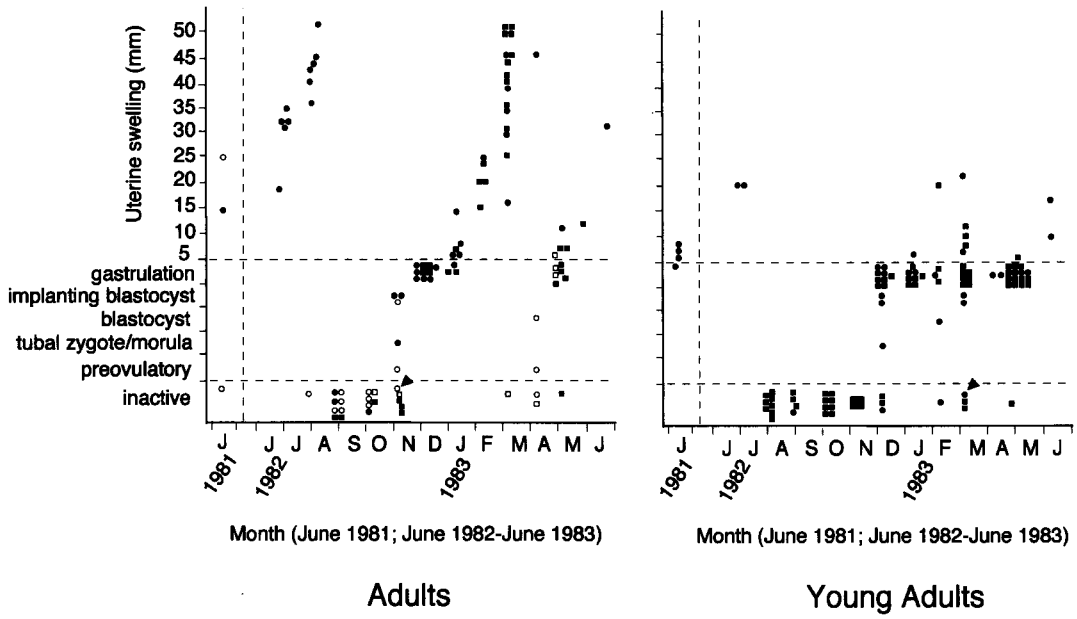

FIG. 2.—Reproductive status and developmental stages of embryos from adult and young adult *P. jagori* from June 1981 and from June 1982 to June 1983. Circles represent females whose reproductive tracts were autopsied; squares represent those marked and released. The large squares represent 15 and 13 individuals in the adult and young adult graphs, respectively. An open symbol indicates lactation; lactation in females with near-term embryos is not shown in the figure. Arrowheads indicate females resorbing an embryo. All marked and released embryos with uterine swellings of 2.5–5 mm are categorized as having gastrulation stage embryos, although it is possible that several did not.

tational region were elongated relative to those of the contralateral uterine horn.

A post-parturient uterine horn was characterized by large diameter (up to 5 mm in preserved specimens) and an irregularly shaped lumen filled with cellular debris and red blood cells, with the surrounding epithelial layer damaged or missing. One female with a tubal blastocyst of ca. 250 cells had a post-parturient uterine horn on the contralateral side. Because this female probably conceived after parturition and development to this stage is likely to require more than 1 week (e.g., Rasweiler, 1979), this suggests that recovery of the post-parturient horn may require at least 1–2 weeks.

Ovulation and fertilization.—Primary, secondary, early antral, and atretic follicles were found in both ovaries in all months. Follicies with larger antra were present in April and in September/October for adult females. Two adults were observed to have large tertiary follicles, one on 5 November 1982 and one on 7 April 1983 (Fig. 1c).

For adult females, ovulation took place in late March and April and then again in October and early November (Fig. 2). For most young adult females, ovulation occurred in November and early December, ca. 1 month later, on average, than ovulation in adults (Fig. 2). Three young females had implanting blastocysts in February and March, suggesting that some individuals ovulate even later. Two of these females each had a single corpus albicans in the contralateral ovary, indicating a probable earlier ovulation that apparently did not result in successful pregnancy. One young female collected in late August had a triovular secondary follicle, suggesting that polyovulation may occur rarely in this species. No other such follicles were observed.

Twins have been observed in an adult female *P. jagori* on Maripipi Island (P. D. Heideman, in litt.).

Spermatozoa were found in uteri of females captured in all months except March, July, and September, suggesting that mating occurred at almost any time of year. However, only 3 of 21 females had sperm in their uteri during the period from late June through October, when no females were captured with early embryos. Presence of spermatozoa in uteri of females that lacked mature pre-ovulatory follicles suggested that mating was not tied closely to ovulation in *P. jagori*. Spermatozoa were found in uteri of six of 10 females that had not ovulated and also lacked large preovulatory follicles. Most females with embryos at early stages of development also had spermatozoa in their uteri. Uteri of three of four females with unimplanted tubal or uterine embryos contained spermatozoa, as did uteri of five of eight females with early implanting embryos, seven of nine females with implanted embryos at various stages of gastrulation, and three of six females with somite-stage embryos. The vagina of one female that was pregnant with a gastrulating embryo contained a firm white mass that appeared to be a sperm plug, and her uterus contained large numbers of spermatozoa. None of six females with embryos in the last third of pregnancy had spermatozoa in their uteri. Only 1 of 11 females that were lactating or had recently given birth had spermatozoa in her uterus, but most of these females ($n = 9$) were captured in July and September when no females were conceiving.

Pregnant females had only a single corpus luteum (Fig. 1b), and ovulation was assumed to have occurred from the ovary containing the corpus luteum. Number of ovulations from the right and left ovaries did not differ significantly (27 right, 31 left; $P > 0.05$). In six of six cases, tertiary follicles or tubal embryos occurred contralateral to a corpus albicans or post-parturient uterine horn. Therefore, it appeared that *P. jagori* exhibited alternating ovulation (6 contralateral to last pregnancy, 0 ipsilateral; $P < 0.05$). Number of ovulations and implantations on each side was not statistically different for young adults (14 right, 9 left; $P > 0.50$) or for adults (13 right, 20 left; $P > 0.50$), and therefore, *P. jagori* does not exhibit ovarian dominance.

All embryos occurred ipsilateral to the corpus luteum. In females with young embryos (<7 mm), corpora lutea took up 50–80% of the ovary as a mass of cells with large amounts of cytoplasm and, in some females with unimplanted embryos, with blood-filled spaces (Fig. 1b). In pregnancies that were near term, corpora lutea showed signs of regression.

Implantation.—Implantation occurred in mid-April and mid-November in adults (Fig. 2). In young adults, the presence of embryos in gastrulation in early December indicated that implantation probably had occurred in November, but young adults with implanting embryos also were observed in December, February and March (Fig. 2).

The zona pellucida surrounded three of three tubal embryos (three morulae at stages of ca. 64, ca. 130, and ca. 250 cells) but was not observed surrounding any preimplantation blastocysts in the uterus. All preimplantation and implanting blastocysts were centered within the progestational region of the uterus, where hypertrophy of the uterus and the proliferation of uterine glands were most extreme (Figs. 1d and 3a).

Initial adhesion of the blastocyst was superficial; the embryo was centered in the progestational region of the uterus and attached around its entire circumference (Figs. 1d and 3a). The preimplantation or earliest implanting blastocyst consisted of an undifferentiated mass of cells surrounded by a layer of columnar trophoblast (Fig. 3a). In places where the developing trophoblast and endometrium were in contact, the uterine epithelium was either degraded or missing (Fig. 3a). By gastrulation, an em-

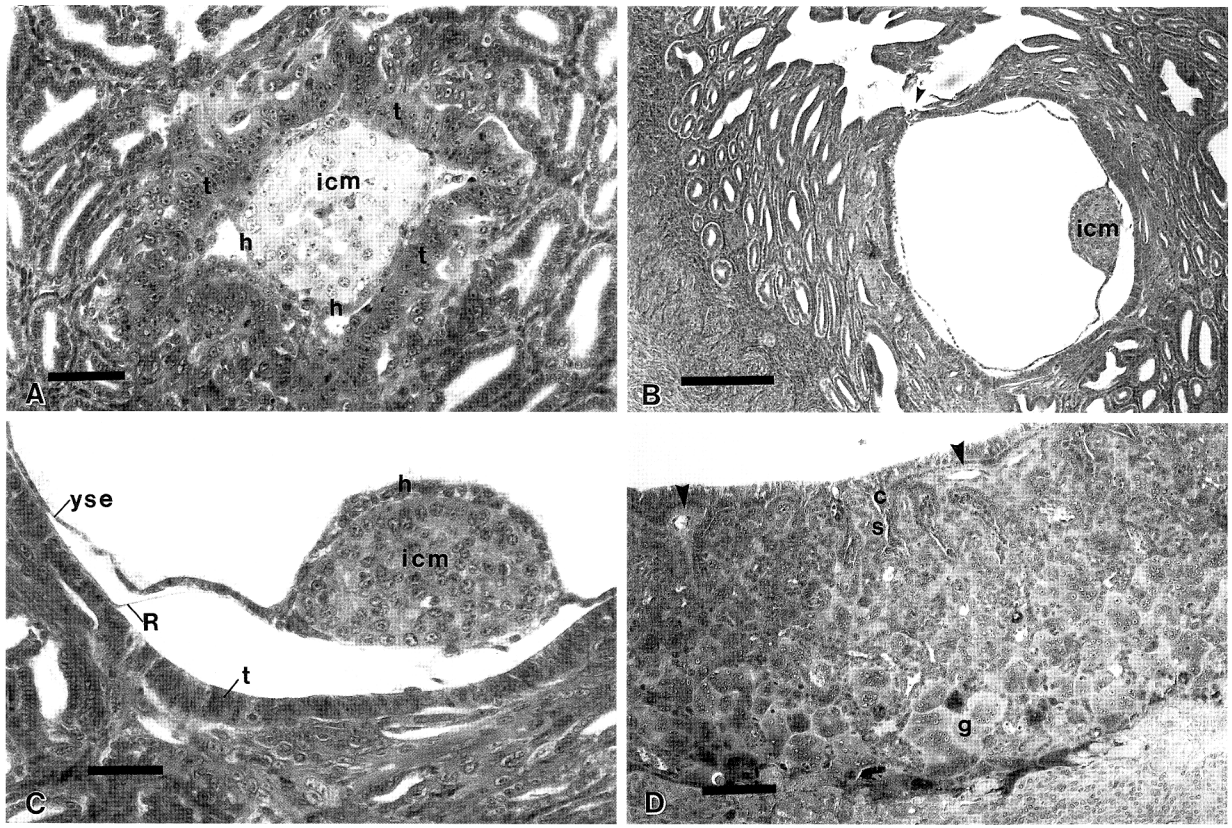

FIG. 3.—A) An early implanting blastocyst (from Fig. 1D at higher magnification) surrounded by a layer of undifferentiated, columnar trophoblast cells (t): h, hypoblast; icm, inner cell mass. Scale bar = 50 μm. B) Implanting blastocyst of an adult on 4 November 1982. Maternal tissue almost completely encloses the embryo to separate it from the uterine lumen. There is a small gap indicated by an arrowhead where undifferentiated trophoblast is in contact with the lumen. Scale bar = 200 μm. C) Higher magnification of the embryo in Fig. 3B shows that the trophoblast (t) has not differentiated and uterine epithelium is degraded where it contacts trophoblast. Yolk sac endoderm (yse) has extended from the hypoblast (h) along Reichart's membrane (R). Scale bar = 50 μm. D) Extraembryonic membranes and uterine tissue from a young adult carrying an embryo in gastrulation on 4 February 1983. The trophoblast of this embryo is completely differentiated into cytotrophoblast (c) and syncytiotrophoblast (s), surrounding maternal blood spaces (arrowheads) and terminates at a layer of maternal multinucleate giant cells (g). Scale bar = 100 μm.

bryo and its trophoblast were embedded completely within the uterine wall, and by late gastrulation, a decidua capsularis had developed, indicating that implantation was secondarily interstitial (Figs. 3b and 3c). One embryo was preserved during late stages of this process (Figs. 3b and 3c); the trophoblast of this embryo was embedded completely in uterine tissue, except for a tiny gap (Fig. 3b, arrow) only a few cells in width that exposed trophoblast to the uterine lumen. Throughout the early period of implantation, the uterine epithelium was eroded by the proliferating trophoblast, which also sent cords of cells into uterine glands. Uterine tissue also hypertrophied during this period, and the resulting growth and, eventually, fusion of tissues over the caudal portion of the trophoblast formed the decidua capsularis. The implanting blastocyst conformed to the shape of the uterine lumen at early stages, but at late stages of implantation, the yolk-sac cavity had expanded to form a large fluid-filled sphere containing the embryo.

By completion of implantation, the trophoblast had differentiated into discrete cytotrophoblast and syncytiotrophoblast layers, which penetrated the uterus to a region containing maternal multinucleate giant cells (Figs. 3d and 4a). The trophoblast had surrounded maternal blood spaces by this stage of development. This stage of implantation was observed as early in development as early gastrulation, before mesoderm induction, and was the case for all embryos at the stage of neurulation.

At early stages of development, embryoblasts tended not to be oriented toward the mesometrium but instead were oriented toward the utero-tubal junction at the cranial end of the uterus. All embryoblasts were at least slightly oriented toward the cranial tip of the uterus, and 21 of 27 cases examined were oriented to within ca. 45° of craniad.

Early development.—Early in implantation, the embryoblast was a roughly spherical mass of cells within the blastocoel. During implantation, the embryoblast developed an outer layer of hypoblast (Fig. 3c). A layer of yolk-sac endoderm extended outward from the hypoblast along Reichert's membrane, eventually forming the yolk-sac membrane lining the yolk-sac cavity. The inner core of cells beneath the hypoblast layer of the embryoblast differentiated into an outer layer of columnar epiblast surrounding a central sphere of cells. At this stage, the embryoblast was a sphere consisting of an outer layer of presumptive endoderm surrounding a layer of presumptive ectoderm, in turn surrounding an inner spherical core of cells. Just before gastrulation, the inner spherical core of cells began to develop a central proamniotic (primary schizamniotic) cavity. At about the same time, a secondary proamniotic (secondary schizamniotic) cavity opened between the presumptive ectoderm and the inner core of cells in the region where the embryoblast was in contact with Reichert's membrane (Fig. 4a). The primary cavity in the center of the inner core of cells expanded as the cells at the cavity boundary degenerated. The secondary cavity expanded as the presumptive ectoderm at its boundaries elongated, enclosing an increasingly larger volume (Fig. 4b). Finally, just before or early in gastrulation, the two schizamniotic cavities fused to form a single proamniotic cavity (Fig. 4c), and the inner sphere of cells in later embryos was entirely missing, while the cavity contained debris.

In embryos during or just after fusion of the two proamniotic cavities, presumptive mesoderm appeared between the presumptive hypoblast and epiblast (Figs. 4c and 4d). In embryos at later stages, this layer of mesoderm was continuous with an extraembryonic mesodermal layer between the yolk-sac endoderm and Reichert's membrane, and, along the boundary of the proamniotic cavity, with a layer between ectoderm and Reichert's membrane.

Annual reproductive timing in adults.—Adult *P. jagori* gave birth to a single offspring twice a year, once in late March or

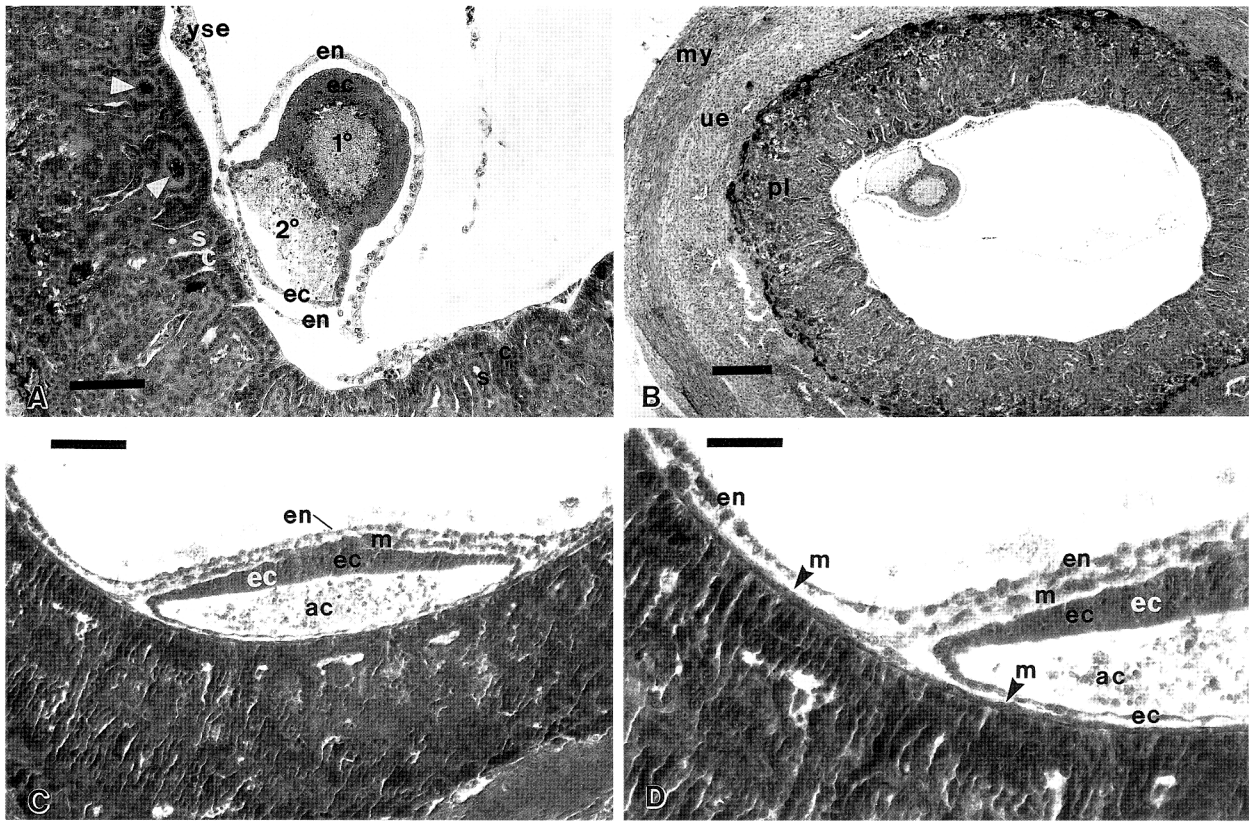

FIG. 4.—A) An embyro at the gastrulation stage from a young adult on 8 January 1983. The embryo consists of ectoderm (ec), endoderm (en), and the primary (1°) and secondary (2°) schizamniotic cavities. The yolk sac endoderm (yse) is also visible. The trophoblast has differentiated into cytotrophoblast (c) and syncytiotrophoblast (s) surrounding maternal blood spaces (arrowheads) containing red blood cells. Scale bar = 100 μm. B) The embryo from Fig. 4A at a lower magnification; my, myometrium; ue, uterine endometrium; pl, developing placenta. Scale bar = 200 μm. C) An embryo at the embryonic disk stage from a young adult on 5 May 1983. The three distinct germ layers, endoderm, mesoderm (m), and ectoderm are present as well as the amniotic cavity (ac). Uterine tissue was damaged due to poor fixation. Scale bar = 100 μm. D) The embryo in Fig. 4C at a higher magnification. The mesoderm extends out from the embryo beneath Reichert's membrane and extraembryonic endoderm and ectoderm (arrowheads point to extraembryonic mesoderm). Scale bar = 50 μm.

early April and once in August (Fig. 2). Following parturition in August, adult females were anestrous for 3 months. They entered estrus around the end of October and early November. In contrast, after parturition in March 1983, most adults were already pregnant by the end of April. We estimated that gestation length in adult females was ca. 4 months, because pregnant adults were first observed in early November 1982, whereas near term and lactating females were first observed in early March 1983.

Small amounts of milky fluid could be expressed from teats of females that were pregnant with near-term embryos in March or in August. One adult post-parturient female was lactating in March, all were lactating in April, and about one-half were lactating in May. Most females were lactating in late August–early September and October, and one-half of the adult females captured in November also were lactating (Fig. 2).

Annual reproductive timing in young females.—Most young females captured during the period from December to May were marked and released. However, reproductive tracts of 18 young females were selected in those months for examination and sectioning, with a particular focus on females that were not palpably pregnant or were of uncertain status (nine of those selected). Every female with a 2.5–5 mm uterine swelling had an embryo in gastrulation, those few with slightly larger uterine swellings held embryos with somites, those with uterine swellings ≤2 mm had implanting embryos or had embryos in gastrulation, and those lacking uterine swellings were either not pregnant or held a tubal morula.

Eight young females held embryos in gastrulation at the stage of amniogenesis, just before induction of the mesoderm and embryonic disc formation (Figs. 4a and 4b). Yolk-sac endoderm, ectoderm, and a proamniotic cavity were present in all. In two of those eight embryos, mesoderm was just beginning to form (Fig. 4d). Six females, in all of which the progestational region of the uterus was ≤2 mm in diameter, had tubal or implanting embryos. Interestingly, each of the three females with preimplantation or implanting embryos in February and March had a corpus albicans in the contralateral ovary, evidence of a previous ovulation. The two young females with uterine swellings of 6 and 7 mm had embryos at the stage of neural tube formation and had somites. Only two of the 18 young females were not pregnant; both lacked mature pre-ovulatory follicles but had spermatozoa in their uteri.

There was evidence that most young females undergo a period of delayed post-implantation development. Young adult females became pregnant as early as late November and December, but almost no young females carried post-gastrulation embryos during the period between then and May. In 49 of 58 cases (84%), pregnant young females had uterine swellings of ≤5 mm in the 6 months from December through May, implying that females either repeatedly lost embryos and were reimpregnated or that their embryos were in some form of developmental delay. We found only one young female with a degenerating embryo during this period in March, suggesting that few females were losing embryos. Additional evidence for a delay in post-implantation development came from marked and recaptured females. Eight young marked pregnant females captured early in this period were recaptured again 40–86 days later; seven had uterine swellings 3–5 mm in diameter, typical of gastrulation-stage embryos, at both captures (Table 1); the eighth female (#T032) apparently was pregnant, gave birth, and became pregnant again. In contrast, this was not true for any of the six full adult females marked and recaptured over the same period (Table 1). Our data suggested that the period of delay for young females ended in May. Five of six pregnant young females examined histologically in June (four in 1981 and two in 1983) and

TABLE 1.—*Young adult and adult females captured twice or three times from November 1982 to May 1983. T032, B10, B9, and B26 all presumably gave birth and became pregnant again, and T439, B24, and T451 were recaptured twice. Note there is no significant increase in embryo size of most young adults, but there is considerable change in embryo size of adult females.*

	Identification number	Capture date	Embryo size (mm)	Recapture date	Embryo size (mm)
Young adults	T440	6 Dec 1982	3	10 Feb 1983	3.5
	T439	6 Dec 1982	2	6 Feb 1983	4.5
				2 May 1983	4
	T458	8 Dec 1982	Not pregnant	4 Mar 1983	22
	T474	9 Dec 1982	Not pregnant	4 Mar 1983	8
	T302	10 Dec 1982	3	19 Jan 1983	5
	T503	24 Dec 1982	Not pregnant	5 May 1983	2.5
	T032	8 Feb 1983	20	2 May 1983	6
	T586	4 Mar 1983	Not pregnant	27 Apr 1983	4
	T591	4 Mar 1983	4	27 Apr 1983	3
	T128	6 Mar 1983	4	28 Apr 1983	3
	T381	7 Mar 1983	4	3 May 1983	4
	B24	7 Mar 1983	5	28 Apr 1983	4
				8 May 1983	4
Adults	T414	8 Nov 1982	Not pregnant	7 Feb 1983	20
	T447	6 Dec 1982	3	12 Jan 1983	6
	T451	7 Dec 1982	4	4 Feb 1983	15
				7 Feb 1983	20
	B10	6 Mar 1983	40	2 May 1983	4
	B9	6 Mar 1983	40	5 May 1983	7
	B26	5 Mar 1983	30	8 May 1983	4

one young female captured in early July carried embryos well past gastrulation.

Our data suggested that embryos of a few young adults may not undergo any delay, because we found three young adult females with embryos at stages beyond gastrulation in January, February, and March that were at similar developmental stages to those of adults at the same period (Fig. 2). These three young females could have given birth during or soon after the period of adult births in late March and April.

Unfortunately, after mid-pregnancy (uterine swellings of 15–20 mm), young adults were difficult or impossible to distinguish from adult females because phalangeal epiphyses were no longer detectable and teats of pregnant females had developed dramatically, presumably in response to the hormonal stimulation accompanying pregnancy. As a result, some young adult females, those in the second half of pregnancy, may have been misrepresented as adults (Fig. 2). However, no births were identified outside of the two adult peaks, so we believe that most young adults gave birth relatively synchronously with the second birth period of adults in August.

Evidence of similar differences in reproductive strategies for adult and young adult *P. jagori* was noted in February and April–July of 1984. In 14 of 15 cases (93%), young adult females caught from February through June had embryos 3–5 mm in size. In contrast, embryos of adults showed evidence of normal development through two birth peaks in 1984, the first in late April and the second in early September (data not shown).

Resorption of embryos.—We observed two instances of degenerating embryos. A lactating adult on 5 November 1982 with no measurable uterine swelling was found to have undifferentiated trophoblast tissue, remnants of Reichart's membrane, embryonic tissue with pycnotic nuclei, and red

blood cells within the progestational uterus. Quantity of tissue and specific types of tissue present in this embryo corresponded to those of an implanting embryo. A non-lactating young adult on 6 March 1983 had a 6-mm uterine swelling with substantial numbers of red blood cells, connective tissue, and cells with pycnotic or missing nuclei filling the uterine lumen. In each of these females, uterine glands and epithelium were normal in appearance elsewhere in the uterus.

Discussion

Reproductive morphology and stages of early development in *P. jagori* were generally similar to those of other cynopterine fruit bats (Heideman, 1989*a*; Heideman et al., 1993; Hood, 1989; Keibel, 1922; Moghe, 1956). Ovaries of most *P. jagori* were subdivided into regions containing predominantly primary follicles, secondary and antral follicles, or atretic follicles intermixed with collapsed zonae pellucidae (Fig. 1a). Ovulation alternated between the two ovaries, as in other megachiropterans (Wimsatt, 1979). A fully developed corpus luteum can double or triple the volume of the ovary. Unlike the situation in *Haplonycteris fischeri* (Heideman, 1989*a*), the corpus luteum persisted throughout pregnancy, and a corpus albicans was sometimes still apparent early in the next pregnancy, although there were signs that the corpus luteum had begun to regress by late pregnancy. The slight uterine progestational reaction (Marshall, 1949; Pow and Martin, 1994) observed in *P. jagori* was similar to that described by Gopalakrishna and Karim (1971) in *Rousettus leschenaulti* and by Heideman (1989*a*) for *H. fischeri.*

As is typical of megachiropterans, there was evidence that mating was not restricted to the short period at ovulation (O'Brien, 1993). Spermatozoa were found in uteri of females lacking mature pre-ovulatory follicles and females with somite-stage embryos weeks after ovulation. Adult females became pregnant soon after parturition in late March or early April. However, mating did not necessarily occur immediately post-partum, because only one of three lactating females with grossly swollen post-parturient uteri in April had spermatozoa in her uterus; the single inseminated female was the only one that also was pregnant (with a tubal zygote). In addition, one female with a vaginal sperm plug, presumably from a recent mating, was pregnant with an embryo in gastrulation. This suggested that females became sexually receptive before ovulation and remained receptive throughout early pregnancy, at least up to the somite stage of development.

As in other pteropodids, implantation in *P. jagori* occured near the cranial tip of the uterus (Heideman, 1989*a*; Rasweiler, 1979). Early embryos were oriented toward the utero-tubal junction at the cranial tip of the uterus, as has been reported in *Otopteropus cartilagonodus* (Heideman et al., 1993) and several other species of bats, including the pteropodid *R. leschenaulti* (Rasweiler, 1979). Initial attachment was superficial, but implantation was secondarily interstitial, as is typical of many chiropterans (Rasweiler, 1979). During the early period of implantation, the uterine epithelium was eroded by the proliferating trophoblast, and cords of trophoblast extended into the uterine glands. Uterine tissue also hypertrophied during this period, and the resulting growth and eventual fusion of tissues over the caudal portion of the trophoblast formed the decidua capsularis.

Implantation and early development in *P. jagori* was similar to that of *H. fischeri* (Heideman, 1989*a*) and *O. cartilagonodus* (Heideman et al., 1993). Completion of implantation was marked by discrete layers of cytotrophoblast and syncytiotrophoblast that terminated at a layer of multinucleate giant cells (Wimsatt, 1951) and was similar to the pattern described for *H. fischeri* (Heideman, 1989*a*). We found subtle differences between early development in the three Philippine species and descriptions of early development in an Indian cynopterine,

Cynopterus sphinx (Gopalakrishna and Karim, 1979; Moghe, 1956). Moghe (1956; and see Gopalakrishna and Karim, 1979) had few specimens undergoing amniogenesis, and from them, suggested that a single proamniotic cavity was divided into primary and secondary amniotic cavities by development and fusion of folds of embryonic ectoderm. They suggested that this layer of ectoderm breaks down shortly thereafter, reforming a single cavity. Our specimens of *P. jagori,* as well as those of other Philippine cynopterines, showed that 1) both cavities are interior to the layer of ectoderm of the embryoblast, 2) the two cavities developed independently in these species, and 3) the two cavities fused following breakdown of the intervening tissue layer. As a result, formation of the amniotic cavity in three Philippine species does not involve development and fusion of dorsal folds of ectoderm. Our examination of the earlier descriptions (Keibel, 1922; Moghe, 1956) suggests that amniogenesis in *Cynopterus* may actually follow the same pattern as that of the Philippine cynopterines. After fusion of the proamniotic cavities in the *Ptenochirus* embryo, a layer of extraembryonic mesoderm was observed between the yolk-sac endoderm and Reichert's membrane. This extraembryonic mesoderm was of uncertain origin; it could have formed in place (Gopalakrishna and Karim, 1979), or may have been produced as extensions of mesoderm migrating from the embryoblast itself.

In young females, far more embryos in gastrulation were found than expected. Embryos of adults required ca. 1 month (4 weeks from mid-November to mid-December) or less (2–3 weeks in late April) to progress through gastrulation, and embryos of gastrulation stage were found only in short periods centered in November and late April–early May (Fig. 2). In contrast, 71% (41 of 58) of pregnant young females captured from early December to early May had uterine swellings of 2.5–5 mm, indicating that they were in gastrulation. All but one of the pregnant young females recaptured over this span had embryos within this size range at both captures. Only one young female captured at this time held remnants of a degenerating embryo, suggesting that this pattern was not due to repeated reabsorption of embryos followed by a second pregnancy. Instead, these data indicate that most young females in this population undergo a delay in post-implantation development that occurs at about the time of gastrulation. In some young adult females, the delay may not occur or is of short duration, because a few young females had mid-term embryos in January and February at similar stages to those of multiparous adults. Normal development of delaying embryos of young adult females resumed in May and June when 7 of 10 young females weighing >68 g had post-gastrulation embryos.

It is possible that parous adult females underwent a short period of delayed development in December. The period around gastrulation appears to have been slightly longer in November–December than in April–May (Fig. 2). Our data do not allow us to resolve this question. However, it is clear that such a delay, if it occurred, lasted no more than a few weeks in parous adults.

Because of a delay of up to 5 months, young females underwent a gestation period that may be up to 9 months in length. Presence of a few females with pre-gastrulation embryos from December to March indicated that young females became pregnant over a span of at least 4 months. This also suggested that the delay was not of a fixed length. As a result, some young females gave birth as early as March (no delay), a few between March–July (variable length of delay), and most in July or August (longest delay in development).

Characteristics of embryos in delay are similar in *P. jagori, H. fischeri,* and *O. cartilagonodus* (Heideman, 1989*a*; Heideman et al., 1993). In all three species, the delay appears to occur during gastrulation, just

before and during the time when mesoderm differentiates and spreads.

Although post-implantation embryonic development can be slowed by reductions in body temperature in many species of bats (Heideman, in press; Racey, 1982), a post-implantational delay at a particular stage of embryonic development has been demonstrated only in a few species of bats (Bradshaw, 1962; Fleming, 1971; Heideman, 1989*a*; Heideman et al., 1993; Rasweiler and Badwaik, 1997) but may occur in several others (Bernard and Meester, 1982; Krishna and Dominic, 1982, 1983; McWilliam, 1987; Wimsatt, 1975). In those species for which histological material is available, there is evidence that embryonic development is nearly or entirely halted for periods of months in or just after gastrulation, shortly after implantation.

The mechanism for these delays is entirely unknown. Although it has been speculated that limits to the nutrient supply to the embryo may inhibit development (A. C. Enders, in litt.), we have found no clear differences in vascularization of uteri holding gastrulating embryos among pteropodids that do and do not undergo delay (P. D. Heideman, in. litt.). We hypothesize that developmental delay may be caused by inhibition of one or more genes crucial for gastrulation or post-gastrulation development. Genes involved would need to inhibit not just growth and development of the embryo itself but also of the extra-embryonic membranes. It seems plausible that such genes could act simply by inhibiting development of mesoderm, which is required for further development of both the embryo and the placenta. We propose that inhibition is regulated by maternal factors and could consist either of the lack of some necessary stimulatory molecular signal or production of an inhibitory signal by the mother. In other species, it has been suggested that delays might be regulated by maternal thyroxine (Burns et al., 1972), progesterone (Burns and Easley, 1977), or prolactin (Richardson, 1979).

Ptenochirus jagori is the first species in which delayed development has been reported to play a role in reproductive timing in one age class, young adults, but not in another age class, adults. It is unclear why embryos of many, but not all, young females undergo delay. It is clear that a long delay period is not obligatory in this species, because adults and, apparently, some young females do not have developmental delays. It is possible that young females use a facultative delay in embryonic development to adjust their reproductive effort in their first year. Late pregnancy and lactation are the most energetically demanding stages of development for females (Racey, 1982). A facultative developmental delay may allow young females to conceive and have the option of giving birth early or postponing late pregnancy and lactation until conditions are optimal. Thus, young females in excellent physical condition (and not under stress—Rasweiler and Badwaik, 1997) in a good year might give birth in March–April and produce a second offspring in July–August. Those that were not in peak condition could give birth only once in their first year, using the delay to postpone parturition until July–August.

Presence of the delay implies that there exist mammalian genes and maternal signals that can halt (or nearly halt) the process of development at the time of gastrulation, a period when a series of complex cell movements, communication, and specification and differentiation must occur to permit normal development. Further study of the delay and its control may help identify processes that regulate gastrulation and also may suggest methods for manipulation of embryos in gastrulation. Widespread occurrence of delayed post-implantational development in bats suggests that factors that permit delay in bats are conserved and may be shared by other groups of mammals.

Ptenochirus jagori is the fourth species and fourth genus of cynopterine bat for which there is evidence of delayed embryonic development (Heideman, 1989*a*; Hei-

deman et al., 1993; Krishna and Dominic, 1983). Cynopterine fruit bats appear to be particularly likely to incorporate developmental delays into their reproductive strategies. In these species, delays are important components of the mechanisms that cause seasonal reproduction. Delays range from 8 months to perhaps as little as a few weeks. Delays may or may not be obligate, and their durations appear to be variable, at least in some species. This suggests that many characteristics of delay may be readily modified by natural selection. In discussions of the potential adaptive significance of reproductive delays in bats, it has been suggested that use of delays may function to increase the ability of females to respond flexibly to conditions in a particular year (Heideman, 1988), may synchronize reproduction in a population (Heideman, 1988; Racey, 1982), and may function as integral components of a strategy of seasonal timing (Bernard, 1989; Heideman, 1988). All of these are potentially applicable to *P. jagori.*

Conservative reproductive strategies among young female bats have been reported in at least three families. Young *Myotis lucifugus* and *Coleura afra* females reproducing for the first time have been reported to resorb embryos under adverse conditions (McWilliam, 1987; Schowalter et al., 1979). Resorption of embryos by *P. jagori* and those other species may allow females to recover quickly from a failed reproductive attempt. Krishna and Dominic (1983) report that some *C. sphinx* young females undergo a longer gestation period, presumably through some form of delay, to synchronize births with older females. There may be advantages for some or most young females of *P. jagori, C. afra,* and *C. sphinx* to produce only one young in their first year, while older, more experienced females normally reproduce twice annually (Krishna and Dominic, 1983; McWilliam, 1987).

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