Hemoglobin Genotype and Fertility in a Malarial Environment: Limon, Costa Rica

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ABSTRACT

This dissertation tests the hypothesis of differential fertility of Hb AS females in a malarial environment. The biocultural factors that affect achieved reproduction in this sample are examined. The aim of the research is to document the action of natural selection through differential fertility, and the interaction of culture and biology to produce achieved fertility.

Epidemiological, clinical and in vitro studies indicate that Hb AS individuals experience low malarial morbidity and mortality. Thus, natural selection increases the frequency of hemoglobin S through differential mortality when the selective agent is malaria. However, it has also been proposed (Livingston, 1957) that the high frequency of hemoglobin S in malarial environments may be the result of differential mortality and reproduction. Firschein's work (1961, 1984) suggests that Hb AS women have higher fertility in a malarial environment because Hb AA mothers suffer more frequent abortions as a result of placental malaria.

Fertility data and blood sample were collected from 159 black women from the coastal town of Limon, Costa Rica. This investigation indicates that there are no significant differences between Hb AS and Hb AA mothers in terms of live births (t=-0.69, ns, df=98), completed family size (t=0.79, ns, df=98) or abortions (t=0.61, ns, df=93).

The analysis of the reproductive data identifies the main biocultural factors that affect achieved fertility in the Limon population. In this sample, age at menarche is significantly correlated with the number of abortions (r=-0.28, p< 0.005) indicating that early menarche increases the risk of fetal wastage. Women with completed family size above average are different from their peers only in the number of pregnancies and livebirths. A path analyses of the fertility data allows the examination of the causal structure of achieved fertility in the sample. The path analyses indicates that the number of multiple births and abortions influence the completed family size mainly through their correlation with the number of pregnancies.

In conclusion, this dissertation does not support the hypothesis of differential reproduction of Hb AS and Hb AA females in a malarial environment. This study confirms prior indications that the Hb S polymorphism has been maintained solely through differential mortality. In contrast with previous research, this dissertation incorporates biological and cultural components of completed fertility. Achieved fertility in Limon is influenced by the age at menarche, the number of pregnancies, and the number of abortions. The age at marriage does not influence the reproductive outcome.
Para mi Papá y mi Mama'. Sin su ayuda, apoyo y amor, esta
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Chapter 1

Introduction

The high frequency of the hemoglobin S gene in human populations has been attributed to natural selection operating through malaria. Since this explanation was first proposed by Haldane in 1946, a considerable body of evidence confirming the malarial connection has been accumulated (Beauvais and Beauvais, 1986).

The "malaria hypothesis" states that the Hb AS heterozygotes are resistant to Plasmodium falciparum infection. In contrast, individuals with hemoglobin A experience mortality as a result of malaria more frequently than do heterozygotes. Thus, natural selection operates through differential mortality on Hb AA and Hb AS individuals. Without medical treatment, most homozygous Hb SS individuals die before reproduction. Since natural selection favors the heterozygote over either homozygote, the Hb S system is considered to be a balanced polymorphism, where the frequency of Hb S is greater than 1% (Allison, 1954, 1956/1957; Beauvais and Beauvais, 1986; Charmot, 1980; Fleming et al. 1985; Guggenmoos-Holzmann et al., 1981; Taylor and Siddiqui, 1982).

The earliest evidence in support of the malaria hypothesis is the geographic concordance of endemic malarial areas and of populations with high frequencies of Hb S (Allison, 1956, 1954, 1956/1957; Beauvais and Beauvais, 1986; Colombo and Felicetti, 1985). This concordance suggests that natural selection has acted upon the polymorphism to maintain these frequencies.
Epidemiological and clinical studies have provided clear evidence in support of the malaria hypothesis. In general, this line of research examines the relationship between parasite density and the incidence of infection in individuals with Hb AS and Hb AA genotypes. The results of these investigations suggest that heterozygotes suffer lower levels of parasitemia in comparison with control groups. Several studies indicate that malaria morbidity is significantly reduced in heterozygotes (Colombo and Felicetti, 1985; Perrin et al., 1982; Taylor and Siddiqui, 1982). Two early studies, (Garlick, 1960; Thompson, 1969) demonstrated the existence of significantly lower P. falciparum parasitic rates among "sickler" as compared to normal children. More recently, Fleming et al., (1985) also reported significantly lower parasitic levels among heterozygous children from 0 to 24 months of age. With only one exception (Ringelhann et al., 1976), all reports have demonstrated lower parasitic rates and morbidity levels among Hb AS individuals of all ages (Colombo and Felicetti, 1985; Fleming et al., 1985; Guggenmoos-Holzmann, 1981; Perrin et al., 1982; Taylor and Siddiqui, 1982; Thompson, 1969). Charmot (1980) notes that acute malarial crises which result in death occur less frequently among heterozygotes.

In vitro studies provide further evidence for the malaria hypothesis. This line of research provides valuable data on the actual cellular mechanism that protects heterozygotes from P. falciparum infection. Early in vitro studies clearly demonstrated
that *P. falciparum* could invade and grow successfully in Hb AS and Hb AA cells if the oxygen tension is maintained at 18%. If, however, the oxygen tension is lowered to 1-5%, infected Hb AS cells sickle rapidly (Taylor and Siddiqui, 1982). Under these conditions, non-parasitized Hb AS erythrocytes sickle more slowly (Luzzatto et al., 1970; Roth et al., 1978). Sickling occurs readily in red blood cells (RBCs) infected with the younger stages of *Plasmodium falciparum*, while host cells infected with older malarial organisms do not sickle (Fleming et al., 1985; Friedman and Trager, 1981). Roth et al. (1978) observed that host cells with older parasites contained polymerized hemoglobin, which inhibits *P. falciparum* growth.

Friedman (1978) suggests that parasite growth lowers intracellular pH and reduces oxygen tension. These factors trigger sickling, which in turn leads to parasite death. The erythrocyte is then able to resume its normal shape (Friedman et al., 1979b). The in vitro observations of other investigators suggest that sickling is not necessary to prevent parasite growth. Pasvol et al. (1978) argue that the heterozygous host cells undergo significant changes which may cause parasite death prior to sickling. Thus, it is possible that a lowered oxygen tension is sufficient for *P. falciparum* to die in an Hb AS erythrocyte. Sickling may be a subsequent and an accessory event (Beauvais and Beauvais, 1986; Charmot, 1980; Pasvol et al., 1978; Tharavanij, 1985).

Geographical, epidemiological and in vitro studies indicate that the high Hb S frequencies in malarial environments are the
result of differential mortality. However, in an early paper, Livingston (1957) proposed that the high frequency of hemoglobin S in malarial environments may be the result of both differential mortality and differential fertility. He suggests that heterozygous females may be buffered from the deleterious effects of malaria during pregnancy.

This dissertation tests the hypothesis of differential reproduction of Hb AS females in a malarial environment. The reproductive careers of a sample of women are analyzed to elucidate the main components of achieved fertility in a natural-fertility population. The specific aims are the following:

1) To determine if the achieved fertility of homozygous Hb AA and heterozygous Hb AS females differs in a black Costa Rican population. The aim is to establish if Hb AS women experience greater achieved reproduction than do their peers under the selective pressure of malaria.

2) To determine the biocultural reproductive factors which influence achieved fertility among a sample of women who use no artificial contraceptive measures.

The second chapter of the dissertation is subdivided into two parts. The first section is a review of previous research on the subject of differential reproduction of Hb AS females. The second section of the chapter reviews the effects of malaria on human pregnancy. This section is a necessary introduction to the test of the differential fertility hypothesis. If it is proposed that heterozygous women enjoy a reproductive advantage in malarial
environments, it must be established that malaria adversely affects the reproduction of Hb AA subjects.

Chapter three contains a description of the materials and methods used in this study, and provides an introduction to the history and demography of the subject population. The fourth chapter contains the results of the study. Tests of differential fertility are presented first, followed by an analysis of the sample's reproductive patterns. Chapter five discusses the results, and chapter 6 the conclusions of this dissertation.
CHAPTER TWO

LITERATURE REVIEW

This chapter examines the importance of malaria as a selective agent through its effects on fertility and the evidence for differential reproduction of Hb AS women as an evolutionary response to the infection. The first section reviews the literature on the reproduction of Hb AS females; the second examines through the literature the effects of malaria on human fertility.

A. THE FERTILITY OF Hb AS WOMEN

MATERNAL MORBIDITY AND MORTALITY

All studies of pregnancy among Hb AS females agree that these women do not constitute a high-risk group obstetrically. The only complication associated with pregnancy among Hb AS females compared to Hb AA gravids is an increased incidence of urinary-tract infections. Whalley et al., (1963, 1964) demonstrated that the incidence of such infections is statistically significant between heterozygotes and homozygous Hb AA controls. This early finding has been replicated in all subsequent studies of pregnant Hb AS women (Fiaspui and Moran, 1973; Gatti e al., 1970; Jennings, 1977; Nouhouayi et al., 1970; Puckett, 1966; Pritchard et al.1973; Renaud and Dervain, 1977). Increased urinary tract infections experienced by Hb S heterozygous females probably result from sickling in the kidneys, organs that provide a hypertonic medium. Sickling may
lead to structural damage and scarring of the kidneys, thus predisposing the patient to renal infection (Blank and Freedman, 1969; Whalley et al., 1963).

In conclusion, pregnant Hb AS women differ from Hb AA controls in terms of morbidity only in their increased incidence of urinary tract infections. This complication does not appear to jeopardize the reproductive performance of Hb AS subjects.

**FETAL OUTCOME**

The literature on the fetal outcome of Hb AS pregnancies reports different frequencies of spontaneous abortions and low-birth weight newborns. For example, Puckett (1966) and Jenkins and Clark (1962) report an increased frequency of fetal wastage among Hb AS gravids. Morrison and Wiser (1976b) report one stillbirth in association with a placenta rich in sickled erythrocytes in a series of 31 pregnancies. Other studies indicate decreased birth weight among newborns of Hb AS subjects. One possible mechanism which accounts for the decreased in utero growth is moderate hypoxic stress (Brown, 1972; Jennings, 1977; Perkins, 1971).

Most studies of fetal outcome among Hb AS gravids fail to demonstrate increased fetal wastage or low birth weight among these subjects (Abrams, 1959; Blank and Freedman, 1969; Desforges and Warth, 1974; Hendrickse and Watson-Williams, 1966; Huntsman, 1976; Morrison and Wiser, 1976b; Pritchard et al., 1973; Renaud and Dervain, 1977; Rucknagel and Larios, 1969). These studies do not indicate that the Hb AS females experience greater fetal wastage, or give birth to low birth-weight neonates significantly more than
do the Hb AA controls.

There are conflicting reports on the fetal outcome of Hb AS pregnancies. Some authors suggest an increased rate of abortions, while others indicate a higher frequency of low birth weight neonates, but most find no difference in either variable between homozygous Hb AA and heterozygous mothers.

STUDIES IN NON-MALARIAL ENVIRONMENTS

Hb AS females in non-malarial environments do not reproduce more or less than do Hb AA women (Blank and Freedman, 1969; Huntsman, 1976; Lambert et al., 1977; Pearson and Vaughan, 1969; Pritchard et al., 1973; Salzano et al., 1973). Brown et al., (1972) found that Hb AS mothers produce low birth weight babies more frequently than do controls, but the total reproductive outcome does not differ.

The most extensive study of the reproduction of Hb AS females in comparison to that of Hb AA women in a non-malarial environment is that by Whalley and collaborators (1963, 1964). The reproductive history of 500 heterozygous and 500 homozygous Hb AA women, ranging in age from less than 20 to over 40 years, was studied. Whalley et al., (1963) found no differences between these homozygous and heterozygous subjects for any reproductive variable or for parity.

In conclusion, research on the reproduction of Hb AS and Hb AA women in non-malarial environments indicates that both groups reproduce equally.
Differential reproduction of Hb AA and Hb AS females in an endemic malarial environment was investigated early by Allison (1956), Delbrouk (1958), Edington (1955), and by Garlick (1960). These reports found no significant difference in the fertility of "sicklers" either in matings with another "sickler" (Allison, 1956) or without regard to the partner's phenotype (Delbrouk, 1958; Edington, 1955; Garlick, 1960). Allison (1956) stresses that his data set indicates that heterosis is due to differential mortality only, and not to differential fertility. The laboratory techniques used to determine the presence of Hb S in these early studies could not precisely identify the various genotypes. This problem complicates the comparison of these early reports with later investigations.

Roberts and Boyo (1960) examined differential reproduction of couples in a malarial environment using electrophoresis to identify the hemoglobin phenotype. The results of this study suggest that the matings of one heterozygote with a homozygous normal are favored by natural selection. However, the fertility differences among these matings are not statistically significant.

Later studies (Firschein, 1961; Hamilton et al., 1972; Custodio and Huntsman, 1984) have approached the problem of differential fertility by examining the reproduction of females without regard to the partner's genotype. The consequences of this change in the experimental design is that few or no comparisons can
be made between Allison's (1956) and Roberts and Boyo's (1960) studies on one hand, and later investigations on the other.

More recently, the fertility of Hb S carriers in a malarial environment was investigated by Firschein (1961, 1984). Using the Black Caribs, Firschein tested the hypothesis of greater fertility of Hb AS females in a malarial environment. He compared the reproductive performance of 89 heterozygotes with that of 254 homozygous Hb AA females. The mean number of children produced by Hb AS women is 6.17, and that of Hb AA females 4.25, a statistically significant difference. Firschein also found a different sex ratio in the families of homozygous and heterozygous subjects, as Hb AA mothers produce fewer males. Since Hb AS women apparently were producing more males, Firschein argues that the frequency of Hb S should be higher in males. He observed this differential sickling rate between the sexes, and attributed it to greater in utero stress experienced by male fetuses.

Firschein (1961, 1984) indicates that, under malarial stress, Hb AS females enjoy greater fertility than do Hb AA women. He proposes that Hb AA mothers experience an excess of abortions due to placental parasitemia. Firschein's study suggests that the high frequency of the sickle cell hemoglobin gene in endemic areas results from both differential mortality and fertility. Firschein (1961) states that among the Black Caribs, selection acts mainly through differential fertility.

Subsequent studies that have tested the hypothesis of differential fertility have failed to replicate Firschein's findings (Custodio and Huntsman, 1984; Hamilton et al., 1972).
Some authors have measured the parasitemia rates in the placenta, while others have used the same population-genetics approach as Firschein.

Custodio and Huntsman (1984) failed to demonstrate any differences in the fertility of homozyous Hb AA and heterozygous subjects among Black Carib groups in endemic malaria areas of Belize and Honduras. Their sample was obtained by door-to-door visits, and not from a gynecological clinic, as did Firschein. Custodio and Huntsman argue that their sample is more likely to represent the entire population, while the clinic sample may be biased. The latter tends to include more "mothers" than does a random sample. Custodio and Huntsman (1984) also failed to demonstrate the different Hb S frequency between the sexes found by Firschein.

Hamilton et al., (1972) have tested the hypothesis of increased Hb AS fertility in Kampala, Uganda, a holoendemic malaria area. Their sample consisted of 231 heterozygous and 1096 homozygous Hb AA mothers with a mean age of 21.67 and a mean parity of 2.35. Neither group of women differed in the mean number of living children, abortions or parity. This extensive study indicated that Hb AS females do not produce more offspring than do Hb AA women. The study by Hamilton and collaborators indicates that even among low-parity young mothers, heterozygotes are not at a reproductive advantage.

The testing of the hypothesis of differential fertility of Hb S heterozygotes in a malarial environment has also been approached
from a different angle. Brabin and Perrin (1985) examined the parasite rates in peripheral blood samples from pregnant Hb AS and Hb AA subjects at different gestational stages. There were no significant differences in prevalence or parasite density between these groups.

Two studies have examined the placenta parasitemia rate in the heterozygous and homozygous subjects. It is of importance to determine if heterozygotes have fewer parasitized placentas than do Hb AA homozygous, because parasitized placentas are the mechanism proposed by Firschein to account for differential reproduction. In an early study, Cannon (1958) observed that the parasite density in placentas of 100 "sicklers" did not differ from those of 206 normal subjects. A more recent study by Jilly (1969) identified hemoglobin phenotypes through electrophoresis. Jilly replicated the results reported by Cannon (1958), namely, that the placentas of Hb AS subjects are equally parasitized as those of Hb AA women. Finally, Fraser (1966) examined the issue of differential fertility by comparing the sibship size of Hb AA and Hb AS males in the Congo. He reports that heterozygous males had non-significantly greater sibships. The experimental design of that study precludes any comparison with other investigations discussed in this section.

To summarize, only Firschein has found significant evidence for increased fertility of Hb AS women in a malarial environment. Previous and subsequent studies have shown that the fertility of heterozygous and homozygous Hb AA females does not differ in a malarial environment. Both groups of women seem to have comparable
numbers of pregnancies, abortions, live births and placental parasitemia.

B. THE EFFECTS OF MALARIA ON HUMAN PREGNANCY

The effects of malaria on pregnant hosts who have not been exposed to infection in an endemic fashion have been well documented. The disease results in high rates of fetal wastage, premature newborns, high neonatal mortality, and even congenital malaria because the mothers lack immunity (McGregor, 1984; McGregor et al., 1983). The effects of endemic malaria on immune, pregnant women is less clear. Malarial endemicity is probably more important evolutionarily because more women have been exposed to the pathogen in this form.

RECRUDESCENCE OF MALARIA DURING PREGNANCY

In immune subjects, malarial parasitemia and its clinical manifestations are exacerbated during pregnancy. In some cases, infection may even result in maternal death (Bianchi and Bergamaschi, 1984; Brabin, 1983; Bruce-Chwatt, 1983; Correa et al., 1982; McGregor, 1983; Playfair, 1982; Sankar et al., 1985; Shaw, 1985; Strang et al., 1984; van Zon and Eling, 1980). Two models have been proposed to account for the recrudescence of malaria during pregnancy. One involves the maternal immune system, and the other the vascular characteristics of the placenta.
Pregnancy is associated with a number of immune changes, some of which decrease the likelihood of fetal rejection by the mother (Anagnos et al., 1986; Bruce-Chwatt, 1983; Loke, 1982; Playfair, 1982; Strang et al., 1984; Sankar et al., 1985). Several studies indicate that the levels of Ig G are significantly lower among pregnant women. Since anti-Plasmodium antibodies are of an Ig G class, pregnant women have lower levels of antibodies to respond to the infection (Bray and Anderson, 1979; Ibeziako et al., 1980; Loke, 1982; Taufa, 1978).

Loke (1982) cautions that the decrease of Ig G during pregnancy may not be the result of immunosuppression, but rather of increased extracellular fluid in the blood associated with the pregnancy. This phenomenon may dilute the concentration of circulating antibodies. Moreover, malarial antibodies cross the placenta, thus lowering their levels in the maternal circulation (Loke, 1982). McGregor et al., (1983) and McGregor (1983, 1984) suggest that the recrudescence of malaria during pregnancy is the result of heavy parasitization of the placenta. This organ is highly vascular, and it may shield infected erythrocytes from the extra-uterine immune system (Anagnos et al., 1986; Watkinson and Rushton, 1983). Heavily parasitized placentae have been reported in immune women who have no Plasmodium in their peripheral blood system, which indicates that the placenta is a privileged site for parasitic growth (Bray and Sinden, 1979; Brabin, 1983; Bruce-Chwatt, 1952; Jilly, 1969; McGregor et al., 1983; McGregor, 1984; Meerstadt, 1986; Playfair, 1982; Taufa, 1978; Vinayak et al.,
Infected host cells have membrane knobs that may affix the erythocytes to endothelial cells. However, some investigations have failed to demonstrate this form of attachment (Bray and Sinden, 1979; Loke, 1982; Taufa, 1978; Taylor and Siddiqui, 1982).

Placental modifications attributed to *P. falciparum* parasites include thickening of the trophoblastic basement membrane, and some pathological changes in the intervillous spaces (Bruce-Chwatt, 1952; Galbraith *et al.*, 1980a; Loke, 1982; Meerstadt, 1986; Strang *et al.*, 1984). Additional placental damage may result from the host's immune response. The intervillous spaces are infiltrated by infected erythrocytes, and by macrophages, monocytes and lymphocytes (Loke, 1982). These aggregates of monocytes and infected RBCs on one hand, and of antibodies and soluble antigens forming immune complexes on the other, may damage the placenta (Bruce-Chwatt, 1952; Galbraith *et al.*, 1978; Galbraith *et al.*, 1980a). Moreover, the malarial pigment haemoglobin tends to accumulate as infected erythrocytes are destroyed, thus causing cellular death at the trophoblast membrane (Galbraith *et al.*, 1980a). This pigment is a complex molecule formed by the parasite as it breaks down the host's hemoglobin (Strang, 1984).

Two models have been proposed to account for the exacerbation of malaria infection during pregnancy. One involves immunosuppression, while the other involves an increased parasitemia of the placenta. However, these explanations are not mutually exclusive.

In conclusion, malaria during pregnancy is usually exacerbated
among immune individuals. The mother may suffer from increased malaria morbidity, thus compromising the fetal outcome.

CHARACTERISTICS OF PREGNANCY THAT INFLUENCE FETAL OUTCOME DURING MALARIAL INFECTION

With few exceptions, most studies indicate that younger mothers have an increased risk of suffering serious complications from malaria during pregnancy (Bah et al., 1982). Older mothers are less affected by placental malaria (Anagnos et al., 1986; Jellife, 1968; McGregor, 1983) or have fewer clinical complications associated with \textit{P. falciparum} infection (Reinhart et al., 1978). This age effect may be confounded by parity, since it is difficult to partition both effects in human populations (Jellife, 1968; Reinhardt et al., 1978). Van Zon and Eling (1980) report an independent effect of parity and maternal age in mice. They note that younger multi and primigravids tend to experience more severe malaria during pregnancy than do older gravids.

The reasons for the differential age effects of malaria on pregnancy are not understood. Although higher antibody titers among older women may be implicated, young mothers have adult-levels of immunity after puberty (Charmot, 1980; Cohen and Butcher, 1972; Taylor and Siddiqui, 1982). Although van Zon and Eling (1980) note that the age and parity effects are independent in mice, this may not apply to humans.

Among pregnant women, parity seems to exert a profound effect on the clinical aspects of malaria. Primigravids experience greater peripheral blood parasitemia (Anagnos, 1986; Brabin, 1983; Mac Gregor, 1983, 1984; Reinhardt, 1978; Sankar et al., 1985; van

The reasons are unclear for the increased clinical severity among primigravids. In an early study, Cannon (1958) suggested that the parity effect is not independent from the age effect, and that older women have greater immunity. However, it is now understood that individuals living in an endemic malarial area achieve premunition by puberty (Charmot, 1980; Cohen and Butcher, 1972). Other studies (Librach, 1979; McGregor, 1984; McGregor et al., 1983) indicate that the reproductive organs of immune primigravids are not prepared to deal with placental parasitemia. After the first pregnancy, the reproductive organs are immune, which in turn lowers placental parasitemia. Thus, McGregor et al., (1983), McGregor (1984) and Watkinson and Rushton (1983) make a distinction between the individual's immune status, and her immunity in the reproductive tract.

Other studies have reported that no relationship exists between parity and parasitemia. Bah et al., (1982), Correa et al., (1982), Ezeoke et al., (1985) and Menon (1972) report no increased parasitemia among primigravids. The reasons are unclear for the discrepancies between these groups of investigations.

Gestational development is another characteristic of pregnancy that influences malarial infection. Several studies indicate that the severity of *P. falciparum* infection does not remain constant.
throughout pregnancy (Brabin, 1983). Although Menon (1972) notes that malaria is most severe during late pregnancy, others have found that the severity is greatest during the second trimester (Bah et al., 1982; Brabin, 1983; Correa et al., 1982; Librach, 1979; McGregor et al., 1984; Sankar et al., 1985). Librach (1974) and McGregor et al., (1984) suggest that the greatest morbidity and mortality risk occurs between the 16th and 24th weeks. The gestational effect on malarial exacerbation has also been demonstrated among pregnant mice (van Zon and Eling, 1980).

In conclusion, malarial morbidity during pregnancy seems to be influenced by several factors. The most important are age, parity and gestational development. Although it has not been possible to fully partition the effects of parity and age, it is clear that young primigravids are at an increased risk of malarial morbidity during the second trimester. Thus, the selective action of malaria through fertility is mostly restricted to young primigravids.

FETAL PROTECTION

Given the high level of placental parasitemia among pregnant immune mothers, it is remarkable that congenital malaria is a rare event in endemic areas. The human placenta is efficient in the protection of the fetus (Logue et al., 1973; McGregor et al., 1983; Taufa, 1978; Williams and McFarlane, 1970).

The first form of fetal protection involves the maternal cell-mediated immunity against P. falciparum. Parasitized placentae are rich in mononuclear phagocytes (Galbraith et al., 1980a; Loke, 1982). Fetuses are also protected from congenital malaria by the transplacental passage of malarial antibodies. With few
exceptions, the children of infected mothers are born with high levels of antibodies (Logue et al., 1973; Omanga et al., 1982; Playfair, 1982; Strang et al., 1984; Williams and McFarlane, 1969). A third factor that protects the fetus from placental parasites is fetal hemoglobin, which does not support P. falciparum growth (Omanga et al., 1982). Thus, if a parasite avoids the humoral and cell-mediated placental immunity, it will find a hostile environment in the fetus (Charmot, 1980; Diggs et al., 1971; Pasvol et al., 1976).

**FETAL OUTCOME**

The primary mechanism through which the malaria organism compromises fetal development is reduced oxygen diffusion. Decreased placental efficiency is due to the sequestration of infected erythrocytes and of the host's lymphocytes and macrophages. An accumulation of these cells restricts blood flow in the placenta (Gilles et al., 1969; Loke, 1982; McGregor et al., 1983; Watkinson and Rushton, 1983). Decreased oxygen supply to the fetus may also be a consequence of maternal anemia (Strang et al., 1984; Taufa, 1978). Watkinson et al., (1985) note that the abnormal accumulation of cells in the placenta may prevent materno-fetal steroid exchange and thus cause fetal wastage. Malaria during pregnancy may also jeopardize the fetal outcome by inducing premature labor or abortion as a consequence of pyrexia (Gilles et al., 1969; Loke, 1982; McGregor et al., 1983; Strang et al. 1984; Taufa, 1978).
Thus, several studies indicate that the placenta's ability to diffuse oxygen and to exchange steroids is compromised by malarial infection. Some researchers suggest that placental parasitemia may result in abortion, intrauterine death and premature labor (Bah et al., 1982; Bianchi and Bergamaschi, 1984; Menon, 1972).

However, it has not been clearly demonstrated that *P. falciparum* infection is associated with fetal wastage among immune mothers. McGregor et al., (1983) argue that the evidence for this association among immune mothers is not unequivocal. Other researchers have also failed to demonstrate greater rates of abortion among parasitized mothers (Anagnos et al., 1986; Gilles et al., 1969; McGregor, 1984). The only negative effect of malaria on reproduction, demonstrated by all studies, is low birth weight (Anagnos et al., 1986; Archivald, 1956; Brabin, 1983; Bruce-Chwatt, 1952; Cannon, 1981 Gilles et al., 1969; Jellife, 1968; Librack, 1979; Loke, 1982; MacGregor and Avery, 1974; McGregor, 1984; McGregor et al., 1983). Low birth weight is one of the most important causes of neonatal death (McGregor et al., 1983).

Malaria can compromise a woman's fertility by congenital transfer of the infection causing subsequent neonatal death. However, the incidence of this among immune mothers is extremely low even when the placenta is heavily parasitized (Louis, 1965; McGregor, 1984). Reinhardt et al., (1978) note that the incidence of congenital malaria in immune populations is 0.03%, while in non-immune individuals it rises to 1-4%. Meerstadt (1986) suggests that transplacental infection may be more common than it is usually thought to be.
This section has reviewed the effects of *Plasmodium* infection on human fertility. Pregnancy exacerbates malarial infection in the immune hosts. Pregnancy-related outbreaks of malaria are more severe among young primigravids. Some authors have demonstrated that after the first pregnancy, mothers rarely experience a malaria crisis. The placenta can be heavily parasitized even if the mother does not experience any malaria morbidity. This parasitation can impair transplacental oxygen diffusion. The effect of impaired placental function on the fetus is not fully understood. Some studies indicate that parasitized placentae are associated with higher fetal wastage and decreased birth weight, while most only report the latter. Congenital malaria is an extremely rare occurrence in endemic areas, and from a population perspective its effects may be of little consequence.

In conclusion, the effects of malaria on fertility are controversial. *P. falciparum* infection may affect young primigravids by inducing fetal wastage, lowering birth weight, or both. Its effects on older multigravids are not understood. As a selective agent during pregnancy, malaria exerts its influence mainly among young primigravids. However, the extent of such action has not been clearly documented.
CHAPTER THREE
MATERIALS AND METHODS

A. THE POPULATION

Limon is the eastern-most province of Costa Rica, comprising one fifth of the national territory. The province extends from the northern national border with Nicaragua, south to Panama. The Limon coastline is 210 kilometers long, and it encompasses the country's entire Atlantic coast (Chavez et al., 1982). Figure 1 shows a map of the country with the province highlighted.

Because Limon has few urban centers, most of the province's population is rural. Sixty-eight percent of the population lives in towns of fewer than 2,000 inhabitants (Chavez et al., 1982). Most population centers are located along the Atlantic railroad track (Mennerick, 1964). Limon city, or Puerto Limon, is the capital of the province, with approximately 30,000 inhabitants, or 26% of the entire province's population (Chavez et al., 1982). The research for this dissertation was conducted in Limon city and six small adjacent towns: Westfalia, Cuba Creek, Matina, Zent and Moin.
Limon has the poorest health care of Costa Rica, with only three well-equipped hospitals for the entire province. The rural population obtains its health care in small clinics, which fails to provide the medical needs of the people (Chavez et al., 1982).

Throughout its history, the principal health hazard of the province has been malaria. Mennerick (1964) indicates that malaria infestation is one of the most important factors responsible for the underdevelopment of the province. The disease precluded the colonization and agricultural development of the vast lowlands, and it was probably the most common cause of death during the railroad construction. Casey (1979) notes that malaria was the most frequent cause of death in the province during the period of the United Fruit Company enclave. In 1939, Kumm and Ruiz carried out a
malaria survey of Costa Rica. Figure 2 shows that the Atlantic and Pacific coasts were endemic areas at the time. *P. falciparum*, *P. vivax* and *P. malariae* were present, and the most common vector was *A. albimanus* (Faust, 1941; Kum and Ruiz, 1939; Watson and Hewitt, 1941).

Figure 2. The distribution of malaria in Costa Rica in 1941. (Kum and Ruiz, 1939)
Kum and Ruiz (1939) palpated 9126 children for spleenomegaly, and obtained blood films for 43.6% of the subjects in the entire country. Since 74.2% of the children with P. falciparum infection had enlarged spleens, the spleenomegaly method underestimates the actual rate of parasitation. In Limon, 11.9% of the children had an enlarged spleen, and 75% of the localities sampled were infested with P. falciparum. Kum and Ruiz's survey (1939) demonstrates that P. falciparum was endemic in Limon.

After the Kum and Ruiz survey, the available data on malarial endemicity in Costa Rica do not partition the number of reported cases by geographical area. However, the study by Kum and Ruiz (1939) clearly shows that the Atlantic and Pacific lowlands were the only endemic malaria areas. Thus, most malaria cases reported in the following tables are likely to have taken place in the Atlantic and Pacific coastal areas. The data concerning malaria endemicity in Costa Rica after 1939 provide various and incomplete information. For example, from 1939 through 1947, the number of malaria deaths was recorded, but not the number of cases. After 1947, the number of cases is available through 1985, but the number of deaths is not always reported.

Table 1 summarizes the number of malaria deaths in the entire country from 1939 through 1946. In 1939, the year of the publication of the Kum and Ruiz survey, there were 843 deaths. The number of malaria deaths increased through 1945, and then decreased slightly to 802 in 1946. These figures indicate that in 1946, the selective action of malaria was nearly as strong as it was in 1939,
when the coastal areas were considered endemic.

Table 1. The number of malaria deaths in Costa Rica: 1939-1946. (Source: WHO, 1952).

<table>
<thead>
<tr>
<th>Year</th>
<th>1939</th>
<th>1940</th>
<th>1941</th>
<th>1942</th>
<th>1943</th>
<th>1944</th>
<th>1945</th>
<th>1946</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria deaths</td>
<td>843</td>
<td>917</td>
<td>942</td>
<td>1223</td>
<td>1128</td>
<td>1012</td>
<td>980</td>
<td>802</td>
</tr>
</tbody>
</table>

Tables 2-5 provide the number of malaria cases reported in Costa Rica every ten years from 1947 through 1985. The sources are listed on each table. An asterisk indicates that two sources do not report the same number of cases for a particular year. Since the disagreement never involved more than 8 cases, an average of both figures is presented in the tables. The number of malaria cases decreased slowly from 12,749 in 1947 to 1,210 in 1964. There was an increase in the number of cases reported during 1965-1967, which was followed by a steady decrease through 1982. In 1983, 1984 and 1985 there was an increase in the number of cases, with 245, 568 and 734 cases respectively. In 1983, several counties of Alajuela and Limon experienced malaria outbreaks, totalling 200 cases (Malaria Action Programme, 1985).

Table 2. The number of malaria cases in Costa Rica: 1947-1954 (Sources: WHO, 1953; 1954a; 1954b; 1955; 1956; 1957).

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<tbody>
<tr>
<td>Number of cases</td>
<td>12749</td>
<td>7472</td>
<td>8729</td>
<td>10068</td>
<td>8797</td>
<td>1903</td>
<td>1407</td>
<td>1368</td>
</tr>
</tbody>
</table>
Table 3. The number of malaria cases in Costa Rica: 1955-1964 (Sources: PAHO, 1962; 1965; WHO, 1965).

<table>
<thead>
<tr>
<th>Year</th>
<th>1955</th>
<th>1956</th>
<th>1957</th>
<th>1958</th>
<th>1959</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>1090</td>
<td>1379</td>
<td>1699</td>
<td>2544</td>
<td>1900</td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Number of cases</td>
<td>1995</td>
<td>1673</td>
<td>1583</td>
<td>1224</td>
<td>1210</td>
</tr>
</tbody>
</table>

Table 4. The number of malaria cases reported in Costa Rica: 1965-1974 (Source: PAHO, 1970; 1978; WHO, 1965; 1972; 1974a; 1975a; 1975b; 1976). An asterisk indicates that two sources report a different number for the same year. An average of both figures is provided.

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<tbody>
<tr>
<td>Number of cases</td>
<td>2563</td>
<td>3046</td>
<td>4418</td>
<td>1133</td>
<td>687</td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Number of cases</td>
<td>353*</td>
<td>261*</td>
<td>160</td>
<td>161</td>
<td>171</td>
</tr>
</tbody>
</table>


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</tr>
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<tbody>
<tr>
<td>Number of cases</td>
<td>297*</td>
<td>488*</td>
<td>217</td>
<td>313</td>
<td>307</td>
<td>---</td>
</tr>
</tbody>
</table>

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<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>168</td>
<td>110</td>
<td>245</td>
<td>568</td>
<td>734</td>
</tr>
</tbody>
</table>
Presently, the Costa Rican Ministry of Health periodically sprays houses with insecticides and carries out epidemiologic surveys throughout the province (Babione, 1966; Garcia-Martin, 1972; Rosero-Bixby, 1985; Tropical Disease Program, 1986a, 1986b). Costa Rica is one of three countries with the highest annual blood examination rate in the Americas (Malaria Action Programme, 1987). Chavez et al. (1982) indicate that eradication of malaria has not been possible mainly because of frequent internal population movements, and because of the heavy migration of infected Nicaraguan workers to the Atlantic coast. The Costa Rican Ministry of health attributes most of the 1979 malaria cases to a particularly heavy rainy season which favored an increase of the malaria vector A. albimanus, and to the migration of infected individuals primarily from Nicaragua (PAHO, 1980). The Malaria Action Programme (1985) also attributes the increase of cases during 1983 to migratory movements, particularly in the northern part of the country. The data presented in Tables 1-5 show that there were at least 1000 cases reported in Costa Rica until 1968. Since the Pacific and the Atlantic lowlands were the only endemic areas in the countries (Kum and Ruiz, 1939), most cases are expected to have taken place in the coastal regions.

A unique trait of Limon is its ethnic diversity (Casey, 1979; Hersfeld, 1978). In 1982, the population was composed of 49% Whites, 46% Blacks, 3% Indians, and 2% Chinese (Chavez et al., 1982). Limon is a distinct cultural center in Costa Rica mainly
because of its black population. Black Limonenses have maintained their own language, religion, and culture (Duncan, 1972; Mennerick, 1964). The Black population of Limon is a product of the immigration of Jamaican workers through the late 1800's to Limon (Casey, 1979; Herzfeld, 1978). The laborers were brought to work for the construction of a railroad from the Central Valley to Limon (Bryce, 1962; Granados and Estrada, 1967; Herzfeld, 1978; Stewart, 1967; Melendez, 1972; Duncan 1972).

In 1871, the Costa Rican government signed a contract with Henry M. Keith, who was able to construct 48 1/2 miles of track, after which he ran out of funds. At that point (1873), Keith's brother, Minor C. Keith, took over the direction of the project (Casey, 1979; Granados and Estrada, 1967; Herzfeld, 1978; Koch, 1975, 1977; Mennerick, 1964). In 1884, the Soto-Keith contract was signed, stipulating that Keith was to complete the track (Casey, 1979; Stewart, 1964). In exchange for this work, Keith was given the "right" to commercially exploit the railroad for 99 years. After that period, those rights would revert to the State. Keith also was awarded 800,000 acres of land for agricultural development on the Atlantic coast (Herzfeld, 1978; Koch, 1975, 1977; Llorente, 1980). Mennerick (1964) observes that the contract was harshly criticized in Costa Rica because it permitted a monopoly on the transportation of agricultural exports to the Atlantic. In 1972 the contract was declared invalid by the Costa Rican government, and the railroad was nationalized (Chavez et al., 1982; Casey, 1979).
One of the main problems encountered by Keith during the railroad construction was a shortage of labor. In 1883, Costa Rica had only 182,073 inhabitants, and unskilled laborers would not work in the Atlantic area for fear of malaria (Fernandez and Mendez, 1973; Herzfeld, 1978; Koch, 1975, 1977; Mennerick, 1962). In 1873, the government ordered the return of Costa Rican workers from the lowlands due to the diseases that they were suffering (Melendez, 1972). As a result, the government authorized Keith to import foreign labor to complete the construction of the railroad (Fernandez and Mendez, 1973). Melendez (1972) reviews extensively the series of migrations of laborers to Limon. He indicates that from 1871 to 1873 the first workers arrived from diverse Caribbean areas such as Panama, Honduras, Curazao, Belize, Cartagena, Aruba, Surinam, and Jamaica. At that point, the Panama canal was under construction, and Keith was not able to obtain sufficient laborers because Lesseps (the builder of the canal) offered better wages. Thus, Keith decided to import Chinese workers, and in 1873, 653 Chinese arrived in Costa Rica (Melendez, 1972). In 1887, when the Panama project was terminated, 762 Italians arrived in Limon, and they were followed in 1888 by 1000 Italian, German, Swedish and Jamaican laborers (Melendez, 1972). By 1889, a total of 4,200 men were working on the railroad, most of whom were foreign-born (Mennerick, 1964).

By far the largest number of migrant workers came from Jamaica. This group permanently changed the ethnic composition and culture of Costa Rica (Fernandez and Mendez, 1973; Granados and Estrada, 1967). The exact number of Jamaican migrants is unknown, but it
may have been as high as 4,000 by 1890 (Fernandez and Mendez, 1973). Duncan (1972) stresses that these migrants had always hoped to return to Jamaica. They were planning to save money and then return after a few years (Herzfeld, 1978). He also emphasizes the existence of a strong anti-Spanish sentiment among the Jamaicans, who considered themselves members of the British empire. Duncan (1972) suggests that Keith took advantage of the pro-British feelings among the Jamaicans, to avoid paying them. Keith made his workers believe that he was British, and reinforced the idea that they were working for the empire. Although the Jamaicans continued to work without pay sometimes for months, they eventually did revolt against Keith in 1887 (Melendez, 1972).

Because the project suffered serious economic set-backs (as early as 1874), and Keith failed to pay the Jamaican workers, most did not return to the island. For that reason, the Government allocated free land to the Jamaicans for cultivation (Melendez, 1972). These plots of land were located along the railroad, which contributed to the present-day population dispersion in Limon. Throughout the province, settlements are located along the railroad (Chaves et al., 1982).

In the late 1870's, Keith started to plant bananas on his land plots. His first harvest was shipped to New Orleans in 1878, and thereafter he rapidly expanded cultivation (Duncan, 1972; Casey, 1979; Granados and Estrada, 1967).

The construction of the Atlantic railroad was officially completed in 1890, following tremendous loss of life from malaria
and accidents. Mennerick (1964) estimates that approximately 20,000 men died during construction. In 1899, Keith and the Boston Fruit Company founded the United Fruit Company, which in following years purchased land in other Central American and Caribbean countries. A subsidiary of the United Fruit Company, the Northern Railway Company, managed the Atlantic railroad from 1905 through 1972 (Herzfeld, 1978; Mennerick, 1964). Once the United Fruit Company and its subsidiaries controlled most of the banana production, the entire transportation system to get the fruit to the port, and its international marketing, a true economic enclave was established (Herzfeld, 1978). Chavez et al. (1982) indicate that this enclave depended entirely on foreign capital for production, transportation, and marketing. The company paid a minuscule amount of its revenue to the government in taxes. With the expansion of the banana production additional labor was needed, resulting in another important migration of Jamaican laborers. By 1927, as a result of the banana boom, there were 17,245 Jamaicans living in Limon (Mennerick, 1964). Although Costa Rican workers still feared going to the Atlantic zone because of malaria, some national workers did migrate to Limon (Chavez et al., 1982). In total, there were about 70,000 workers in banana-related jobs, and the main employer was the United Fruit Company (Fernandez and Mendez, 1973).

During the 1930's, the situation in Limon province changed radically. First, because of the misuse of the land, the soil was depleted and the banana production diminished considerably. More important, during the first part of the decade the crop was
attacked by two banana diseases, one named "Panama", and the other Sigatoka. The United Fruit Company could not overcome these problems and decided to cease production, thus firing the great majority of the working population in the province (Chavez et al., 1982; Casey, 1979; Mennerick, 1964). At the same time, the company negotiated a new contract with the government and continued to grow banana on the Pacific coast. Fernandez and Mendez (1973) review the political struggle that took place when the United Fruit Company requested to move to the Pacific coast.

When the company left Limon, the province was left in a profound economic depression. The Costa Rican workers either followed the United Fruit Company or looked for work in other areas. But for the Jamaicans the situation was much worse. First, they had no money to return to their homeland as they had planned. Second, because they were not Costa Ricans, they could not seek employment out of those jobs stipulated by the initial contract between the United Fruit Company and the government (Mennerick, 1964). Bryce (1962) notes that some Jamaicans were able to migrate to Panama. By 1950 the black population of Limon had decreased 23.9% from the 1927 population size (Fernandez and Mendez, 1973). The remaining workers turned to subsistence farming in the abandoned farms. The situation was so difficult in Limon, that local officials and Costa Rican workers complained to the National government about the presence of foreign labor (Casey, 1979). In following years, more stringent laws were passed prohibiting the hiring of foreigners in Costa Rica except in the Atlantic coast.
region (Fernandez and Mendez, 1973). For a few years (around 1946) the situation was ameliorated because of increases in cacao prices, which prompted the population to raise this crop. When the cacao prices dropped, the population was again in a state of economic depression (Bryce, 1962; Mennerick, 1964).

The 1948 National Constitution signaled the beginning of the incorporation of the black population into the country (Herzfeld, 1978). This constitution legally recognized the once-Jamaicans as Costa Rican citizens, and incorporated laws against racial discrimination (Fernandez and Mendez, 1973). Starting that year, there was a great influx of Blacks Limonenses to the cities of the Central Valley, in search of better jobs and education. Since 1960, a number of public institutions have been created to develop the Atlantic area and to provide jobs for the unskilled workers of the former plantations (Chavez et al., 1982). Notwithstanding these efforts, Limon remains relatively underdeveloped compared with the rest of Costa Rica.

The culture of the black population sharply contrasts with that of the remainder of the country. Bryce (1962) points out that the black Limonenses belong to the western Caribbean cultural area rather than to the Latin American cultural area.

The family structure of the Limon inhabitants in general, and of the black population in particular, is different from that of the predominantly Latin national population (Herzfeld, 1978). The black family is characterized by unstable male-female unions, and a relatively low frequency of marriage. Thus, while the national average of couples living in common-law unions is 4.6%, it is 12.2%
in Limon (Chavez et al., 1982). Not only are common-law unions more frequent in Limon, but these unions are more unstable than marriages.

Black families frequently form consanguineal households, which include members of the extended family (Brown, 1975; Gonzalez, 1969; 1984; Olwig, 1981; Whitehead, 1984). Headley and Sandino (1983) report that in their survey of Puerto Limon families, 5.6% of the black households include relatives outside the nuclear family, while only 3.4% of white households are extended. During the course of the present investigation, it was determined that most household members not belonging to the nuclear family were part of the mother's family. Within the mother's family, the strongest ties are found among the females, which results in a powerful support group of women. Several of the households surveyed were composed of a grandmother, her middle-aged daughters, their daughters of reproductive age and younger children of both sexes. Thus, the Limon households show a marked tendency towards matrifocality. The family structure of the Limon black population closely resembles previously-described domestic units among various black groups in the Caribbean region. Several ethnographic studies report frequent female-headed units, unstable conjugal unions, frequent consanguineal households, high illegitimacy rates, strong female networks, and matrifocality (Brown, 1975; Gonzalez, 1969; 1984; Olwig, 1981; Whitehead, 1978).

An important fertility determinant is the age of the women at the beginning of their reproductive careers. Sanchez (1970)
reports in her survey that out of the 15-19 female cohort, only 34.4% remained single. Thus, 65.6% of these females were already married, living in a common-law union or had already terminated their unions.

The migration to and from Limon is another demographic variable of interest. The well-educated Limonenses leave the province seeking better jobs in the central valley in general, and in San Jose in particular. There has also been a continuous migration of Limonenses to the US (Chavez et al., 1982; Mennerick, 1964). At the same time, many unskilled workers from other regions of Costa Rica migrate to Limon because of job openings in the shipping and agricultural industries. Panamanian and Nicaraguan workers also migrate to Limon. Because the province borders both countries, many unskilled laborers enter the country illegally (Chavez et al., 1982). In a recent survey, it was determined that 53% of a sample from Puerto Limon were born in other areas of the country or in other countries (Headley and Sandino, 1983).
B. METHODS.

Fertility data and blood samples were collected from 159 black women residing in the province of Limon, Costa Rica. All females were considered blacks by the investigator, her laboratory assistant, and by the subjects themselves. Half of the data were randomly obtained during door-to-door visits in Puerto Limon and in rural adjacent towns. The remaining 50% of the samples were collected at the outpatient clinical-laboratory service of the Limon Hospital. These subjects attended the laboratory for non-gynecological reasons. Approximately 10% of the black households in Puerto Limon were sampled.

The primary social distinction between the subjects is urban versus rural place of residence, although only 29 women from adjacent towns were surveyed. At the time of the interview, all rural towns had daily public transportation to Puerto Limon, and all bus rides were less than 1 hour long. No marked socio-economic differences among the subjects were observed, and the sample is homogeneous culturally.

The genotypes of four carriers and two homozygous Hb SS women were known before the samples were taken. Otherwise, sampling for the research had no known bias. The blood samples were obtained by venopuncture by a laboratory technician of the Limon Hospital. The hemoglobin phenotypes were determined by starch gel electrophoresis at alkaline pH followed by a solubility test. For each subject, the G-6-PD phenotype was also established by starch gel electrophoresis. Both tests were performed by the
Investigation Center of Abnormal Hemoglobins and Related Diseases (CIHATA) of the University of Costa Rica. The hemoglobin genotypic and gene frequencies for the entire sample are computed. The frequencies obtained in this study are compared to those reported in Limon and Jamaica by previous research. All analysis on the fertility data are performed on a subsample of 108 women who are older than 49 years of age. This age limitation allows the computation of achieved fertility (Andorka, 1978; Knodel, 1983; Pollard et al., 1981; Pressat, 1978).

The fertility data set consists of the following information:

1. Age at menarche
2. Age at first pregnancy
3. Length of the interval between pregnancies, and age at each pregnancy
4. Outcome of each pregnancy: abortion, miscarriage, live birth
5. Outcome of each live birth: neonatal, childhood, adult death or survival to the time of interview
6. Number of multiple pregnancies
7. Age at menopause.

During the interview, the number of the subjects' siblings, and the female's place of birth were also established. No subjects admitted to the use of contraception at any time in their lives. When possible, additional information was collected on the subjects' menstrual cycle regularity and on the causes of abortions and of the deaths of offspring. The interviews of most women 60 years and older were carried out in English, and those of younger
subjects in Spanish. The place of birth of urban and rural females is analyzed to determine the number of subjects who were born in a place different from their residence at the time of the interview.

In order to quantify the reliability of the subjects' answers, a randomly selected group of 28 women were re-interviewed by another investigator. The females re-interviewed were randomly selected out of a list of 75 subjects who had been interviewed in Puerto Limon at a home visit, and not at the Hospital. The addresses of these women were well known to the author, and could easily and accurately be conveyed to the "follow-up" researcher. The streets in Limon are not numbered, and addresses are given in reference to a landmark. Very frequently, subjects interviewed in the hospital gave as their address their neighborhood, and stated that everybody knew where they lived. In contrast with the author, who is a member of the wider Costa Rican society, the second researcher is a member of the Limon Black community. Both interviewers are female anthropologists. The concordance of both answers for all variables is presented as the percentage of identical responses obtained in both interviews. An analysis of variance (ANOVA) is performed on the answers in order to partition:

1) The error resulting from the different interviewers and,
2) The random error, resulting from different answers provided by the subjects (Committee on population and demography, 1981; Fleiss, 1986).

The significance of the interviewer error is tested by the ratio of the interviewer mean square (IMS) to the error mean square
The random error is quantified by the EMS, and the degree of reliability for each variable is quantified by an intraclass correlation coefficient (R).

The hypothesis of differential fertility is tested by analyzing the reproductive careers of homozygous (Hb AA) and heterozygous (Hb AS) females. The mean completed family size, the number of live births, abortions, and total pregnancies of both groups are compared. Student's t-tests are performed to determine if any of these reproductive variables are different between the Hb AA and the Hb AS subjects (Sokal and Rohlf, 1981). In this comparison, the completed family size is the most important variable because it measures the subjects' achieved fertility.

A discriminant function analysis is applied to the fertility data to determine if it is possible to separate the normal homozygotes from heterozygotes with one or more linear combinations of the original variables. These combinations are known as the discriminant functions, and they maximize group differences. The advantage of this procedure over the univariate t-tests is that the discriminant function analysis combines the information of multiple variables. Thus, if from a wide reproductive view, Hb AA and Hb AS females are different, this analysis should successfully separate both groups (Manley, 1986; Morrison, 1976).

The fertility data set is further analyzed with univariate, multiple regression, path, and principal component analyses. The aim of these tests is to determine which sociocultural reproductive variables influence the achieved fertility in the Limon sample. It is of importance to isolate such variables because achieved
fertility is the variable of most importance in the test of differential fertility of Hb AA and Hb AS females.

An exploratory path analysis of the relationships among the reproductive variables is performed on the entire sample and on the Hb AA and Hb AS subsamples. Emphasis is placed upon the quantification of the direct and indirect effects of the independent variables on completed fertility (Li, 1975; Sokal and Rohlf, 1981).

A stepwise multiple regression procedure is applied to the reproductive data. The aim of the analysis is to predict the achieved fertility of women in the Limon setting. In the model, the dependent variable (Y) is completed family size. A stepwise procedure is used because it may exclude an independent variable from the model to produce the best combination of predictors. The final model is chosen based on the following criteria:

1. A minimized Mean Square error
2. All significant independent variables are in the model
3. All variables in the model contribute significantly to the prediction of Y.
4. A high coefficient of multiple determination ($R^2$), which estimates the proportion of the variation of Y explained by the model.

The final set of variables maximizes the model's predictive value with the minimum possible number of independent variables (Draper and Smith, 1981; Sokal and Rohlf, 1981).

The fertility data set is further examined with a principal
component analysis. The purpose of this procedure is to combine the information of several variables into fewer uncorrelated indices known as principal components. The observed variation of the original variables is adequately and succinctly described by two or three principal components (Manley, 1986; Morrison, 1976). This analysis is chosen because several reproductive variables are highly correlated (Manley, 1986). These variables are: the number of pregnancies, abortions, live births, and completed family size. All statistical analysis are performed with SAS (SAS Institute Inc., 1983) and MINITAB (Ryan, et al., 1982).
CHAPTER FOUR

RESULTS

In comparison with other areas of Costa Rica, the migration rates of Limonenses are very high. Headley and Sandino report that 53% of a sample from Puerto Limon were born in other areas of the country or in other countries. The place of birth of the subjects who participated in this study is presented in Tables 6 and 7. Nine subjects did not report their place of birth.

Table 6. The place of birth of subjects who lived in Puerto Limon at the time of the interview (N=121).

<table>
<thead>
<tr>
<th>Place of birth</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jamaica</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other Costa Rican provinces</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Other towns in the Limon province</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Puerto Limon</td>
<td>61</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 6 shows the place of birth for the women who resided in Puerto Limon at the time of the interview. Fifty percent of the Puerto Limon subjects were born in that town, while 33% of the women were born in other towns of the Limon province. Nicaragua is a more common place of birth (11%) than are other Costa Rican provinces (5%). One female was born in Jamaica.
Fifty-two percent of the females from the rural towns were born in other towns of the Limon Province. Twenty-four percent of the rural subjects were born in the town in which they resided at the time of the interview, and 14% in other Costa Rican provinces. One female was born in Nicaragua, and two were born in Jamaica. These data suggest that rural subjects more frequently are immigrants than are urban females. However, because of the small rural sample size (N=29), it is not possible to determine if the differences between the rural and urban females reflect distinct migration patterns. In concurrence with Headley and Sandino's survey (1983), 50% of the Puerto Limon subjects, and 76% of the rural subjects were born in an area different from that of their residence at the time of the interview. The data indicate that 50% of the Puerto Limon subjects are immigrants.

Table 8 summarizes the incidence of various hemoglobin genotypes identified for a population sample from Limon. Six
blood specimens were lost during transportation and their genotype could not be determined. The following genotypes were obtained: 115 AA, 2 SS, 30 AS, 2 SC, 3 AC and 1 SF. Individuals with hereditary persistence of Hb F have been sampled previously in the Limon population (Saenz, 1987; personal communication). Motulsky (1973) notes that the frequency of the Hb SF genotype in US blacks at birth is 1:25,000. Hb SF individuals tend to have 70% Hb S and 30% Hb F, and they are usually phenotypically normal (Bunn et al., 1977; Feingold et al., 1985; Lehmann and Huntsman, 1974). The Limon Hb SF subject did not experience any morbidity associated with her genotype.

The frequency of the Hb AS genotype is higher in this study than the incidence previously reported by researchers in the same area (Rivera, 1967; Saenz et al. (1971). This discrepancy may be due to the fact that 4 subjects were known carriers, and they were purposely sampled to determine their fertility. The two homozygous Hb SS individuals were also known sickle-cell anemic patients of an unusually mild disease course. Saenz et al. (1984) report 0.06% heterozygotes among 280 newborns of both sexes from Limon. Among 368 females ranging in age from 1 month to 85 years of age, Saenz et al. (1971) find an Hb AS frequency of 8.2%. Rivera (1967) reports a 20% frequency of heterozygotes among 10 black women and 13% among 22 black men and women. In the latter study the high frequency of heterozygotes is probably due to Rivera's small sample.
Table 8. Gene frequencies, and observed and expected hemoglobin genotypes in a sample from Limon (including and excluding the 4 known carriers).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AS</td>
<td>SS</td>
</tr>
<tr>
<td>p²</td>
<td>2pq</td>
<td>q²</td>
</tr>
<tr>
<td>Observed</td>
<td>115</td>
<td>30</td>
</tr>
<tr>
<td>genotypes</td>
<td>75%</td>
<td>19.7%</td>
</tr>
<tr>
<td>Expected</td>
<td>113.2</td>
<td>32</td>
</tr>
<tr>
<td>Gene</td>
<td>frequencies p= 0.86</td>
<td>q=0.12</td>
</tr>
</tbody>
</table>

\[ \chi^2=4.78, \ 1 \ df, \ p<0.05. \]

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AS</td>
<td>SS</td>
</tr>
<tr>
<td>p²</td>
<td>2pq</td>
<td>q²</td>
</tr>
<tr>
<td>Observed</td>
<td>115</td>
<td>26</td>
</tr>
<tr>
<td>genotypes</td>
<td>77.18%</td>
<td>17.45%</td>
</tr>
<tr>
<td>Expected</td>
<td>113.0</td>
<td>28.65</td>
</tr>
<tr>
<td>Gene</td>
<td>frequencies p=0.87</td>
<td>q=0.11</td>
</tr>
</tbody>
</table>

\[ \chi^2=6.42, \ 1 \ df, \ p<0.025. \]
Table 9. The frequency of the Hb AA and Hb AS genotypes among older versus younger women than the average age (X = 56.0)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number observed</th>
<th>Percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>53</td>
<td>72.60</td>
<td>72.60</td>
</tr>
<tr>
<td>AS</td>
<td>20</td>
<td>27.40</td>
<td>100.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>62</td>
<td>86.11</td>
<td>86.11</td>
</tr>
<tr>
<td>AS</td>
<td>10</td>
<td>13.89</td>
<td>100.00</td>
</tr>
</tbody>
</table>

χ² = 4.031  p < 0.045  df = 1

The frequency of the Hb S gene among the subjects is further investigated by dividing the sample into groups of women older and younger than the sample average. Table 9 shows that 27% of the younger women are heterozygous, while only 13.8% of the older women are. These results suggest that the selective pressure of malaria acted upon the older as well as the younger women. Once malaria is removed as a selective agent, heterozygotes loose their advantage over the homozygous normal and the frequency of the Hb S should decrease and eventually return to the mutation rate. However, sampling error in the present study may account for the Hb S frequency difference between both age subgroups, and no conclusions can be drawn until a larger sample is available.

Gatti et al., (1970) demonstrated a decrease in the frequency of Hb S from 1952 to 1969 in Kinshasa, Democratic Republic of Congo, a previously malarial area. Over 1200 subjects were sampled in both
surveys. Gatti et al., (1970) suggest that this decrease is the result of the absence of natural selection operating on the hemoglobin system (Gatti et al., 1970).

Table 8 shows the gene frequencies and expected genotypic frequencies for the Limon sample (Li, 1976). A $\chi^2$ test of the expected and observed genotypic frequencies yields a significant statistic ($\chi^2 = 4.78$, 1 df, $p<0.05$), which indicates that the observed frequencies are not at equilibrium. This test supports the suggestion that natural selection has acted upon the subjects (Li, 1976). It is unlikely that the 4 Hb AS subjects who were sampled because their genotype was known account for this disequilibrium. The expected number of Hb AS females is higher (32) than that observed (30). However, another $\chi^2$ test is applied to the observed and expected gene frequencies without including the four known carriers. The second test also yields a significant statistic ($\chi^2 = 6.42$, 1 df, $p<0.025$), confirming that the observed frequencies are not at equilibrium.

Since most Black Limonenses are the descendents of the Jamaican laborers who migrated to Costa Rica, it is of interest to compare the genotypic frequency of Costa Rican and Jamaican samples. In the Limon sample, the frequency of heterozygotes even without the four known carriers, is higher than that reported in Jamaica (17.45%). With one exception (Saenz et al., 1984), other studies in Limon also report Hb AS frequencies as high or higher than those of Jamaican samples (Saenz et al., 1971; Rivera, 1967). In Jamaica, the following Hb AS frequencies have been
In order to quantify the reliability of the subjects' answers, another investigator asked the same questions of a random sample of 28 women previously interviewed by the author. Table 10 shows the percentage of identical answers obtained by the two interviewers. The answers about the number of siblings are identical in only 17% of the responses. The lack of concordance probably results from the family structure in the Limon Black population. Because of the high instability of male-female unions, subjects are likely to have different siblings who share the same mother, father, and both. Thus, in different interviews, some subjects may report as siblings only those brothers and sisters with the same parents, or mother as their own. The other least reliable variables concern the age at menarche (80% concordant), a topic that may be affected by memory loss, and the number of live births (80% concordant) and abortions (83% concordant). The errors in the number of abortions and live births are related to each other. Four subjects reported the outcome of a pregnancy as a live birth with immediate death of the child in one interview, but they reported the outcome of the same pregnancy as a stillbirth in the other interview. The answers about the age at first pregnancy is concordant for 86% of the subjects, and the number of surviving children and the number of multiple births are concordant for 100% of the women. These results strongly indicate that the most reliable measure for a woman's fertility is her completed family size, not the number of live births she has produced.
Table 10. Percentage of identical answers obtained from 28 subjects by two different interviewers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Agree</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of siblings</td>
<td>17%</td>
<td>83%</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>Number of live births</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>83%</td>
<td>17%</td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>86%</td>
<td>14%</td>
</tr>
<tr>
<td>Number of surviving children</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Number of multiple births</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

An analysis of variance (ANOVA) is applied to the answers of the variables with the lowest concordance, namely, the number of siblings, the age at menarche, the number of live births, and the number of abortions. The aim of the test is to partition the effect of the investigators on the subjects, from the effects of random error. Thus, the model for these ANOVA's assumes that an observation $X_{ij}$ from subject $i$ obtained by interviewer $j$ can be partitioned into:

$$X_{ij} = T_i + p_j + e_{ij}$$

where:

- $T_i =$ the subject's error free score
- $p_j =$ the interviewers effect
- $e_{ij} =$ the random error.
The significance of the interviewers' effect is tested by dividing the interviewer mean square (IMS) by the error mean square (EMS). The critical value is provided by the $F$ distribution, with degrees of freedom $k-1$ and $(N-1)(k-1)$, where $k$ = the number of interviewers, and $N$ = the number of subjects (Fleiss, 1986).

Non-identical responses may be caused by factors such as memory loss, lack of interest in the interview, communication difficulties between the interviewer and the subject, and different time spent in the interview process. This within-subject variance is estimated by the EMS, which is the component of variance due to random error (Fleiss, 1986). The reliability of each variable is estimated by an intraclass correlation coefficient ($R$), a measure of similarity between the answers of each subject. $R$ equals the proportion of the variance of a variable due to subject-to-subject variability in error-free scores (Fleiss, 1986). If the value of the intraclass correlation coefficient reaches its maximum value of unity, there is no variance within each subject's responses, and the variance among subjects is due to subject-to-subject error-free differences. If $R$ equals 0, the answers are unreliable, and the differences among subjects are due to random error (Pleis, 1986; Sokal and Rohlf, 1981).

The intraclass correlation coefficient is estimated as follows:

$$R = \frac{N(\text{SMS}-\text{EMS})}{(N)(\text{SMS})+(k-1)(\text{IMS})+(N-1)(k-1)\text{EMS}}$$

Where:

$\text{SMS} =$ the subject mean square
EMS = the error mean square
IMS = the interviewer mean square
N = the number of subjects
k = the number of interviewers (Fleiss, 1986).

Fleiss (1986) indicates that no simple method is available for constructing a confidence interval for R with this experimental design. Tables 11-14 show the analysis of variance for the number of siblings, abortions, the age at menarche, and the number of live births. Each Table includes a test of significance for the interviewer effect, and the intraclass correlation coefficient (R).

Table 11. Analysis of variance for the number of siblings

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>27</td>
<td>407.05</td>
<td>15.08</td>
</tr>
<tr>
<td>Interviewers</td>
<td>1</td>
<td>6.45</td>
<td>6.45</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>231.05</td>
<td>8.56</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>644.55</td>
<td></td>
</tr>
</tbody>
</table>

Interviewer effect: H0: \( p_1 = p_2 = 0 \). F= 0.75 ns

Intraclass correlation coefficient (R): 0.28

Table 11 shows that the subjects' responses concerning the number of siblings are not significantly influenced by the two different interviewers. However, the reliability estimate is low (R=0.28), indicating that the number of siblings should not be used in the analysis. Table 12 shows the analysis of variance for the
age at menarche. The interviewer effect is not significant, and the reliability measure is high (R=0.81).

Table 12. Analysis of variance for the age at menarche.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>27</td>
<td>134.339</td>
<td>4.976</td>
</tr>
<tr>
<td>Interviewers</td>
<td>1</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>14.482</td>
<td>0.536</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>148.839</td>
<td></td>
</tr>
</tbody>
</table>

Interviewer effect: $H_0: p_1 = p_2 = 0. F= 0.03$ ns
R= 0.81

The analysis of variance for the number of abortions is shown in Table 13. The interviewer effect is not different from 0, and the reliability of this variable is very high (R = 0.87).

Table 13. Analysis of variance for the number of abortions.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>27</td>
<td>57.714</td>
<td>2.138</td>
</tr>
<tr>
<td>Interviewers</td>
<td>1</td>
<td>0.071</td>
<td>0.071</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>3.929</td>
<td>0.146</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>61.714</td>
<td></td>
</tr>
</tbody>
</table>

Interviewer effect: $H_0: p_1 = p_2 = 0. F= 0.48$ ns
R= 0.87
Table 14 shows the analysis of variance for the number of live births. The effect of the interviewers is not significant, and the reliability of this variable equals 0.94.

Table 14. Analysis of variance for the number of live births.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>27</td>
<td>418.054</td>
<td>15.483</td>
</tr>
<tr>
<td>Interviewers</td>
<td>1</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>12.482</td>
<td>0.462</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>430.554</td>
<td></td>
</tr>
</tbody>
</table>

Interviewer effect: \( H_0: \pi_1 = \pi_2 = 0 \). \( F = 0.03 \) ns \( R = 0.94 \)

The ANOVA tests show that the two interviewers did not significantly affect the subjects' responses for any of the variables tested. Thus, the interviewer effect can be assumed to equal 0, and further ANOVA tests can be applied to the same responses. The model for these tests assumes that an observation \( X_i \) from subject \( i \), consists of:

\[ X_i = T_i + e_i \]

The aims of these analyses are:

1) To re-compute \( R \). The intra-class correlation coefficient in this experimental design is estimated as follows:

\[ R = \frac{SMS - EMS}{SMS + (k-1)(EMS)} \]

2) To estimate a one-sided \( 100(1-\alpha)\% \) confidence interval for \( R \), as follows:
\[
R > \frac{\text{SWS}}{\text{EMS}} - \left( \frac{1}{N-1} \right) \frac{\text{SWS}^2}{\text{EMS}^2} + \left( \frac{1}{N-1} \right)
\]

If the confidence interval includes 0, then the answers are unreliable, and the differences among subjects are due to random error. Thus, the null hypothesis that \( R = 0 \) is tested with the confidence interval.

Table 15 shows the reliability estimate (R) and its lower limit for the number of siblings, the age at menarche, the number of abortions, and the number of live births. For purpose of comparison, the previously estimated R is included in Table 8.

Table 15. The reliability estimate (R) for the reproductive variables assuming two different models, and R's confidence interval for the simple model.

<table>
<thead>
<tr>
<th>Reproductive variable</th>
<th>Model ( X_{ij} = T_i + p_j + e_{ij} )</th>
<th>R</th>
<th>R &gt; (Ho: R=0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of siblings</td>
<td>( X_i = T_i + e_i )</td>
<td>0.28</td>
<td>-0.04</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>( X_{ij} = T_i + p_j + e_{ij} )</td>
<td>0.81</td>
<td>0.64</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>( X_i = T_i + e_i )</td>
<td>0.87</td>
<td>0.76</td>
</tr>
<tr>
<td>Number of live births</td>
<td>( X_{ij} = T_i + p_j + e_{ij} )</td>
<td>0.94</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table 15 shows that the reliability estimates for all variables are the same for both models because the interviewer
effect does not differ from 0. The confidence interval clearly indicates that the reliability of the subjects' responses about the number of siblings does not differ from 0. However, the confidence interval of the other reproductive variables is significantly different from 0, and indicates that the data set can be confidently analyzed.

Further analyses are performed on a sample of 108 women, all of whom are older than 49 years of age. Since the rate of childbearing approaches 0 at age 49, it is possible to measure the total number of pregnancies, abortions, live births and the completed family size of the subjects (Andorka, 1978; Knodel, 1983; Pollard et al., 1974; Pressat, 1978).

The descriptive statistics for the sample are shown in Table 16. The mean age of the subjects is 64 years. The mean age at menarche is 14.17 years, a late onset of menstruation when compared to present-day averages in developed countries. The mean age at first pregnancy is 19.42 years, and the mean total number of pregnancies is 6.85. The average number of abortions experienced by subjects is 0.68, and the mean number of live births is 6.22. At the time of this survey, the average completed family size is 5.03. The mean age at menopause is 50.05 years.
Table 16. The descriptive statistics for the 108 females 50 years and older who participated in this study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.0</td>
<td>9.12</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>Age at Menarche</td>
<td>14.17</td>
<td>1.88</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>19.42</td>
<td>4.11</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>Total number of pregnancies</td>
<td>6.85</td>
<td>4.53</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Abortions</td>
<td>0.68</td>
<td>1.16</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td>0.12</td>
<td>0.43</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Live births</td>
<td>6.22</td>
<td>4.29</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Completed family size</td>
<td>5.03</td>
<td>3.72</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>50.05</td>
<td>4.67</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

The subjects' age at marriage is not included in the analyses because in Limon the age at marriage does not mark the initiation of reproduction for the majority of the subjects. Table 17 shows the percentage of women who married before, after, and at the same year of their first pregnancy, and those who never married, among a random subsample of 27 subjects. Among this subsample, only 26% of the women married before their first pregnancy. For the majority of subjects, marriage is not a prerequisite for the initiation of reproduction.
Table 17. The relationship between marriage and the first pregnancy in a random subsample of 27 subjects from Limon.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married the same year of first pregnancy</td>
<td>8</td>
<td>29.5</td>
</tr>
<tr>
<td>Married before the first pregnancy</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Married after the first pregnancy</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Never married</td>
<td>5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

The hypothesis of differential fertility is tested with t-tests and discriminant function analysis. The question examined is do Hb AS females have a reproductive advantage over Hb AA women? Two samples are analysed, one of females 50 years and older, and a larger sample of subjects above 39 years of age. The group of females, above 49 years of age, includes 84 Hb AA and 16 Hb AS females, while the remaining 8 subjects have different genotypes. The larger sample (108 Hb AA, 22 Hb AS) increases the sample size and is utilized to compare the mean values of reproductive variables obtained by the present study with the data of Custodio and Huntsman (1984) and Firschein (1961; 1984).

Table 18 compares the reproductive history of Hb AA and Hb AS subjects 50 years and older. Student's t-tests are applied to determine if any reproductive variable is significantly different between both groups. The t scores for the mean number of live births and for the age at first pregnancy were determined by a test of equality of the means because the variances of both groups are
unequal for these two variables (Sokal and Rohlf, 1981). Since the differential fertility hypothesis states that Hb AS females are expected to have greater achieved reproduction than are Hb AA women, a one-tailed t-test can be performed. The critical t value for alpha=0.1, and 0.05 are checked for the one-tailed and the two-tailed tests respectively. The alternative hypothesis for the first one states that Hb AS females have greater fertility than Hb AA women, and the alternative hypothesis for the second one states that both groups have different achieved reproduction. The null hypothesis for both tests is that the two groups of women are not different reproductively. Table 18 shows that Hb AS and Hb AA women do not differ in the mean number of live births, surviving children, abortions, total pregnancies and multiple births and the mean age at menarche, and first pregnancy. All of the t-scores are clearly not significant for the one-tailed and the two-tailed tests. Thus, the null hypothesis can not be rejected. The completed family size is particularly important because this is 100% reliable variable for the subjects who were reinterviewed (Table 10). Also, the completed family size measures the subjects' achieved fertility at the time of the interview.
Table 18. A comparison of the reproductive performance of Hb AA and Hb AS females for subjects 50 years and older.

<table>
<thead>
<tr>
<th>Reproductive variables</th>
<th>Genotypes</th>
<th>AA (N=84)</th>
<th>AS (N=16)</th>
<th>t</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of pregnancies</td>
<td></td>
<td>7.05</td>
<td>6.00</td>
<td>0.82</td>
<td>ns</td>
<td>93</td>
</tr>
<tr>
<td>Mean number of multiple births</td>
<td></td>
<td>0.07</td>
<td>0.20</td>
<td>-0.85</td>
<td>ns</td>
<td>95</td>
</tr>
<tr>
<td>Mean number of live births</td>
<td></td>
<td>6.36</td>
<td>5.56</td>
<td>0.69</td>
<td>ns</td>
<td>20</td>
</tr>
<tr>
<td>Mean completed family size</td>
<td></td>
<td>5.17</td>
<td>4.31</td>
<td>0.79</td>
<td>ns</td>
<td>98</td>
</tr>
<tr>
<td>Mean number of abortions</td>
<td></td>
<td>0.73</td>
<td>0.53</td>
<td>0.61</td>
<td>ns</td>
<td>93</td>
</tr>
<tr>
<td>Mean age at menarche</td>
<td></td>
<td>14.07</td>
<td>14.57</td>
<td>-0.87</td>
<td>ns</td>
<td>81</td>
</tr>
<tr>
<td>Mean age at first pregnancy</td>
<td></td>
<td>19.70</td>
<td>18.38</td>
<td>1.03</td>
<td>0.3</td>
<td>78</td>
</tr>
</tbody>
</table>

The sample size for the test of the differential fertility hypothesis can be increased by including women younger than 50 years of age. Such inclusion also allows a comparison with the data of Firschein (1961) and Custodio and Huntsman (1984). These researchers did not confine their data set to women older than 49 years of age. Table 19 summarizes from the 3 studies the mean number of live births, surviving children and abortions for Hb AS and Hb AA females older than 39 years of age. These three variables are the only ones analysed in the other 2 studies. However, Firschein does not provide the mean number of abortions in his sample. The data of this study for Hb AA females are similar...
to that reported by Custodio and Huntsman (1984) for the three variables. In contrast, Firschein reports lower means of live births and surviving children. For the Hb AS subjects the mean number of live births and surviving children reported by Firschein is higher than that of the other 2 studies. A comparison of the three data sets indicates that Firschein obtained lower mean values for the Hb AA women, and higher means for the Hb AS females than did the other two studies. The values reported by Custodio and Huntsman and by the present study are similar.
Table 19. A comparison of the Limon data with the data of Firschein (1961) and Custodio and Huntsman (1984). Subjects 40 years and older included.

<table>
<thead>
<tr>
<th>Reproductive variables</th>
<th>Firschein</th>
<th>Custodio and Huntsman</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Hb AA subjects surveyed</td>
<td>145</td>
<td>57</td>
<td>108</td>
</tr>
<tr>
<td>Mean number of live births (Hb AA mothers)</td>
<td>4.89</td>
<td>6.14</td>
<td>6.12</td>
</tr>
<tr>
<td>Mean completed family size (Hb AA mothers)</td>
<td>3.39</td>
<td>5.30</td>
<td>5.00</td>
</tr>
<tr>
<td>Mean number of abortions (Hb AA mothers)</td>
<td>---</td>
<td>0.82</td>
<td>0.72</td>
</tr>
<tr>
<td>Number of Hb AS subjects surveyed</td>
<td>50</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Mean number of live births (Hb AS mothers)</td>
<td>7.04</td>
<td>5.0</td>
<td>5.77</td>
</tr>
<tr>
<td>Mean completed family size (Hb AS mothers)</td>
<td>5.24</td>
<td>3.9</td>
<td>4.68</td>
</tr>
<tr>
<td>Mean number of abortions (Hb AS mothers)</td>
<td>---</td>
<td>0.73</td>
<td>0.66</td>
</tr>
</tbody>
</table>

The difference in the reproductive performance of Hb AS and Hb AA females is tested again, including all subjects above 39 years of age. This test is performed with the aim of increasing the sample size, which now includes 22 Hb AS and 108 Hb AA subjects. Table 20 indicates that the two groups are not significantly different for any variable. The t score for the number of multiple pregnancies was estimated with a test of
equality of the means because the variances of both groups are unequal for these two variables (Sokal and Rohlf, 1981). The increase in the sample size does not demonstrate any reproductive advantage of Hb AS females in the Limon sample. All t scores are below 1, and they are clearly not significant for the one-tailed or the two-tailed tests. The t scores obtained with the larger sample can not be compared with those of the other researchers because Firschein (1961) does not report them, and Custodio and Huntsman (1984) do not apply a statistical test to their data.

Table 20. A comparison of the reproductive performance of Hb AA and Hb AS females. All subjects above 39 years of age are included.

<table>
<thead>
<tr>
<th>Reproductive variables</th>
<th>Genotypes</th>
<th>t</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of pregnancies</td>
<td>AA (N=108) AS (N=22)</td>
<td>0.34</td>
<td>ns</td>
<td>123</td>
</tr>
<tr>
<td>Mean number of multiple births</td>
<td>0.08 0.14</td>
<td>-0.53</td>
<td>ns</td>
<td>23</td>
</tr>
<tr>
<td>Mean number of live births</td>
<td>6.12 5.77</td>
<td>0.36</td>
<td>ns</td>
<td>128</td>
</tr>
<tr>
<td>Mean completed family size</td>
<td>5.00 4.68</td>
<td>0.38</td>
<td>ns</td>
<td>128</td>
</tr>
<tr>
<td>Mean number of abortions</td>
<td>0.72 0.66</td>
<td>0.20</td>
<td>ns</td>
<td>123</td>
</tr>
<tr>
<td>Mean age at menarche</td>
<td>14.13 14.25</td>
<td>-0.25</td>
<td>ns</td>
<td>110</td>
</tr>
</tbody>
</table>

A stepwise discriminant function analysis of the reproductive variables listed in Table 16 is performed. The aim of the test is
to produce multivariate functions capable of discriminating between Hb AA and Hb AS females. This analysis complements the previous univariate analysis, since the reproductive careers of both groups are now examined from a multivariate perspective. If Hb AA and Hb AS females differ in several reproductive variables, the discriminant function analysis should differentiate both groups. A stepwise procedure is applied because it has the capacity of deleting variables from the model if a more informative combination of variables is obtained. However, this analysis fails to yield a function capable of differentiating Hb AA from Hb AS subjects, either when females above 39 or 49 years of age are included. No variable contributes significantly to the discrimination, and none are entered into the model. From a multivariate perspective, the homozygous Hb AA and the heterozygous women do not differ reproductively, thus confirming the results of the univariate analysis.

The biocultural reproductive variables that influence achieved fertility are isolated with univariate, multivariate and path analyses. The primary question examined by these tests is which maternal traits influence completed family size. This variable is the most important one in the test of the differential fertility hypothesis because completed family size is a measure of the subjects' achieved reproduction. The age at marriage, a cultural variable that may influence completed family size in some cultures, is not included in the analysis because marriage does not mark the beginning of the reproductive careers in Limon (Table 17).
Path analyses are performed on the reproductive data for Hb AS, Hb AA and all subjects. These analyses are aimed at partitioning the correlations between the independent and dependent variables into direct and indirect effects. A path analytical model yields an in-depth examination of the effects of maternal traits on achieved fertility. Thus, the correlation between the dependent variable and an independent variable includes:

1. The correlation of the independent variable with other predictors in the model, and

2. The direct effect of the independent variable on the dependent variable, controlling for the other predictors. A path coefficient quantifies this direct effect.

The purpose of path analysis is to provide a more detailed examination of correlation coefficients of the fertility data. This analysis also yields a coefficient of determination (R²) that quantifies the amount of variance of completed family size explained by the maternal reproductive traits in the model. The path coefficients are obtained by standardizing the data and regressing completed family size on the independent variables. Only the diagrams for the Hb AA and Hb AS females are presented because the diagram for the entire sample is virtually identical to that of the heterozygotes. The analyses yield models that incorporate the number of pregnancies, abortions, and multiple births as independent variables. The final models are chosen on the basis of the following criteria:

1. The model has a large coefficient of determination (R²)
with the fewest independent variables.

2. The computed correlations approximate the observed correlations among the variables (Li, 1975; Sokal and Rohlf, 1981). The observed correlations are calculated for both subsamples, and they differ from each other.

Figure 3 is the path diagram for the Hb AA subjects. The equations for the calculated correlation coefficients and their solutions are shown in Table 21. The path diagram demonstrates that the correlation between completed family size and other reproductive variables can be partitioned into direct and indirect effects. Thus, the correlation between the number of pregnancies and the completed family size incorporates the direct path from number of pregnancies to completed family size ($p_{03} = 0.97$). Also, the correlation includes the indirect effects of abortions and multiple pregnancies on the dependent variable. The positive effects of multiple births on completed family size are both direct ($p_{01} = 0.06$) and indirect through their correlation with total pregnancies. Finally, the variable, number of abortions, has a negative impact on fertility ($p_{02} = -0.34$) However, the correlation between abortions and number of pregnancies is also included in the correlation between abortions and completed family size.

This model's coefficient of determination indicates that 86% of the variance of completed family size in the Hb AA sample can be explained by the three reproductive variables included in the model. The residual path probably includes childhood mortality, nutrition, economic status and other factors that affect completed fertility. One of the computed correlations is the same as, and
two are virtually identical to, the observed correlations for the homozygous A females. Thus, the model fits the data well.

Figure 3. A path diagram of the fertility data for Hb AA subjects.
Table 21. Equations and solutions of the path diagram in Figure 3.

<table>
<thead>
<tr>
<th>OBSERVED</th>
<th>COMPUTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_{01} = p_{01} + r_{13} p_{03} = 0.04 )</td>
<td>0.03</td>
</tr>
<tr>
<td>( r_{02} = p_{02} + r_{23} p_{03} = -0.09 )</td>
<td>-0.09</td>
</tr>
<tr>
<td>( r_{03} = r_{13} p_{01} + r_{23} p_{02} + p_{03} = 0.86 )</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\[ p_{01} = 0.06 \quad p_{02} = -0.34 \quad p_{03} = 0.97 \]

\[ R^2 = (p_{01})(r_{01}) + (p_{02})(r_{02}) + (p_{03})(r_{03}) = 0.86\% \]

\[ u = \sqrt{1-R^2} = 0.36 \]

Figure 4 is the path diagram for the Hb AS heterozygous subjects. The equations for the computed correlation coefficients and their solutions are shown in Table 22. The path diagram for the Hb AS females is virtually identical to that of Hb AA subjects except for the inclusion of the correlation between multiple births and abortions, as shown in Figure 4. The correlation between both variables is higher among the carriers \( r_{12} = -0.24 \) than among the homozygotes AA \( r_{12} = -0.05 \), and it is closer to the correlation in the total sample \( r_{12} = -0.11 \). The difference between the correlation coefficient of the Hb AA subsample on one hand, and of the carrier and the total sample on the other is probably due to random sampling of multiple births between both subsets. The mean number of multiple births of the Hb AS subsample is 0.2, and for the Hb AA group it is 0.07. This difference is not significant.

The path analysis successfully partitions the observed positive correlation between completed family size and multiple
births for the entire sample and for the Hb AS subjects. Such correlations result mostly from the indirect positive effects of multiple births on the total number of pregnancies, and from the indirect negative effects of multiple births on abortions.

Table 22 shows that for the carrier subsample, two of the three calculated correlations are identical, and the other one is close to the observed values. The coefficient of determination indicates that 86% of the variance of completed family size in the Hb AS subsample is explained by the three maternal traits.

Figure 4. A path diagram of the fertility data for Hb AS subjects.
Table 22. Equations and solution of the path diagram in Figure 4.

<table>
<thead>
<tr>
<th>OBSERVED</th>
<th>COMPUTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{01} =$</td>
<td>$p_{01} + r_{12} p_{12} + r_{13} p_{03} =$</td>
</tr>
<tr>
<td>$r_{02} =$</td>
<td>$r_{12} p_{01} + p_{12} + r_{23} p_{03} =$</td>
</tr>
<tr>
<td>$r_{03} =$</td>
<td>$r_{13} p_{01} + r_{23} p_{02} + p_{03} =$</td>
</tr>
<tr>
<td>$p_{01} =$</td>
<td>-0.089</td>
</tr>
<tr>
<td>$p_{03} =$</td>
<td>1.08</td>
</tr>
<tr>
<td>$u =$</td>
<td>0.36</td>
</tr>
</tbody>
</table>

The path analyses of the total sample, the Hb AA and Hb AS subsamples, indicate that the relations among the reproductive variables of carriers more closely approximate those of the entire sample than do those of the Hb AA women. These results contradicts the hypothesis of differential fertility. Since 75% of the total sample consists of homozygous A females, both the total sample and the Hb AA subsample are expected to more closely resemble each other than either one is expected to resemble the heterozygous subsample. However, the path analytical model of the total sample is more similar to that of the carrier than to that of the Hb AA subsample. The homozygous A subset differs from the total and the heterozygous samples in the value of the correlation coefficient between abortions and multiple births, and in the value of the path between multiple births and completed family size. The three path analyses strongly suggest that there are no differences in the fertility patterns that influence achieved fertility of homozygous A and heterozygous subjects.

Since all previous tests indicate that Hb AA and Hb AS females
do not differ reproductively, the fertility data set is subsequently analysed without dividing it by genotype. The question examined in all following tests is which biocultural reproductive variables influence achieved fertility. The most important variable to test the differential fertility hypothesis is completed family size because this variable measures the achieved fertility of Hb AA and Hb AS subjects. Thus, it is of interest to isolate the biocultural reproductive factors that influence complete reproduction in the Limon sample.

Table 23 shows the correlation matrix of the subjects' reproductive variables. The total number of pregnancies and of live births are highly correlated ($r=0.95$, $p<0.001$), indicating that both variables should not be included in the multiple regression model to avoid collinearity.
Table 23. Correlation matrix of the reproductive variables for women older than 49 years of age. The rows and columns of this matrix are symmetrical.

<table>
<thead>
<tr>
<th>Variables</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed family size</td>
<td>1.00</td>
<td>-0.03</td>
<td>0.91</td>
<td>-0.05</td>
<td>-0.21</td>
<td>0.09</td>
<td>0.87</td>
<td>0.11</td>
</tr>
<tr>
<td>(A) p&lt; 0.00</td>
<td>0.00</td>
<td>0.78</td>
<td>0.0001</td>
<td>0.58</td>
<td>0.04</td>
<td>0.33</td>
<td>0.0001</td>
<td>0.49</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>1.00</td>
<td>0.04</td>
<td>-0.28</td>
<td>-0.10</td>
<td>-0.11</td>
<td>0.31</td>
<td>-0.24</td>
<td></td>
</tr>
<tr>
<td>(B) p&lt; 0.00</td>
<td>0.00</td>
<td>0.66</td>
<td>0.007</td>
<td>0.32</td>
<td>0.25</td>
<td>0.001</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Number of live births</td>
<td>1.00</td>
<td>-0.11</td>
<td>-0.24</td>
<td>0.18</td>
<td>0.95</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C) p&lt; 0.00</td>
<td>0.00</td>
<td>0.26</td>
<td>0.02</td>
<td>0.05</td>
<td>0.0001</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at Menarche</td>
<td>1.00</td>
<td>0.08</td>
<td>0.20</td>
<td>-0.19</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D) p&lt; 0.00</td>
<td>0.00</td>
<td>0.44</td>
<td>0.05</td>
<td>0.06</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>1.00</td>
<td>0.03</td>
<td>-0.26</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E) p&lt; 0.00</td>
<td>0.00</td>
<td>0.76</td>
<td>0.01</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of multiple births</td>
<td>1.00</td>
<td>0.02</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(F) p&lt; 0.00</td>
<td>0.00</td>
<td>0.80</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>1.00</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(G) p&lt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at menopause</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H) p&lt; 0.00</td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The correlation between the number of abortions and the age at menarche indicates that women who experience early menses tend to experience more abortions. Although the coefficient is moderate (r=-0.28), it is significant at the 0.005 level. This association
has been reported by previous authors (Bracken et al., 1985; Martin et al., 1983) in two United States samples. For purposes of comparison with other studies, the correlation between the age at menarche and abortions is further explored by dividing the subjects into two groups. Group 1 includes those who experienced menarche earlier than the population average (\(\bar{X}=14.17\)), and group 2 includes those who experienced first menses later than the population mean. Table 24 indicates that the mean number of abortions of groups 1 (\(\bar{X}=0.88\)) and 2 (\(\bar{X}=0.35\)) are significantly different at the 0.009 level. However, the two groups do not differ in the mean completed family size. The women in group 1 have a non-significantly greater number of pregnancies, which may compensate for their more frequent abortions. Table 24 indicates that the significantly greater number of abortions experienced by group 1 does not seem to influence the achieved fertility.

Table 24. A comparison of the reproductive performance of women with early menarche (Group 1) and late menarche (group 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>t</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at menarche</td>
<td>12.87</td>
<td>15.77</td>
<td>-11.19</td>
<td>0.0001</td>
<td>87</td>
</tr>
<tr>
<td>Mean age at 1st pregnancy</td>
<td>19.37</td>
<td>19.50</td>
<td>-0.14</td>
<td>ns</td>
<td>85</td>
</tr>
<tr>
<td>Mean number of pregnancies</td>
<td>7.07</td>
<td>6.48</td>
<td>0.63</td>
<td>ns</td>
<td>100</td>
</tr>
<tr>
<td>Mean completed family size</td>
<td>4.86</td>
<td>5.32</td>
<td>-0.61</td>
<td>ns</td>
<td>106</td>
</tr>
<tr>
<td>Mean number of abortions</td>
<td>0.88</td>
<td>0.35</td>
<td>2.65</td>
<td>0.009</td>
<td>100</td>
</tr>
</tbody>
</table>
The differences in age at menarche among the subjects may be related to their age cohorts. By dividing the women into those older and younger than the mean age at the time of the interview (X=64), a significant difference in the age at menarche between both groups is observed. Table 25 shows that women younger than 64 experienced their first menses at a mean age of 13.65, and those older than 64 first menstruate at a mean age of 14.74. The difference between both means is significant at the 0.003 level. Both groups in Table 25 do not differ in their age at first pregnancy, and the number of pregnancies, abortions and live births.

Table 25. A comparison of the reproductive performance of women younger (Group 1) and older (Group 2) than the sample average age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>t</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>55.84</td>
<td>71.57</td>
<td>-17.91</td>
<td>0.0001</td>
<td>101</td>
</tr>
<tr>
<td>Mean age at first pregnancy</td>
<td>19.63</td>
<td>19.17</td>
<td>0.52</td>
<td>ns</td>
<td>85</td>
</tr>
<tr>
<td>Mean number of pregnancies</td>
<td>7.57</td>
<td>6.18</td>
<td>1.54</td>
<td>0.12</td>
<td>100</td>
</tr>
<tr>
<td>Mean number of abortions</td>
<td>0.63</td>
<td>0.73</td>
<td>-0.44</td>
<td>ns</td>
<td>100</td>
</tr>
<tr>
<td>Mean number of live births</td>
<td>6.88</td>
<td>5.60</td>
<td>1.55</td>
<td>0.12</td>
<td>106</td>
</tr>
</tbody>
</table>

In order to determine the main differences between women who produce more or fewer children than the sample average, the
entire sample is further divided into women with completed family size above and below average. Table 26 shows that both groups can be distinguished only in terms of the number of live births and the number of pregnancies. Groups 1 and 2 from Table 26 do not differ in the number of abortions, multiple births, the age at menarche and the age at first pregnancy. The correlation between age at first pregnancy and the number of pregnancies from the correlation matrix in Table 23 (r=-0.26, p<0.01) is not confirmed by the analysis in Table 26. Women who have greater achieved fertility do not start reproduction earlier but experience more pregnancies than do their peers.

Table 26. A comparison of the reproductive performance of women with completed family sizes below average (Group 1) and above average (Group 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>t</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean completed family size</td>
<td>2.57</td>
<td>8.90</td>
<td>-15.46</td>
<td>0.0001</td>
<td>106</td>
</tr>
<tr>
<td>Mean number of abortions</td>
<td>0.71</td>
<td>0.64</td>
<td>0.30</td>
<td>ns</td>
<td>100</td>
</tr>
<tr>
<td>Mean number of multiple births</td>
<td>0.09</td>
<td>0.17</td>
<td>-0.89</td>
<td>ns</td>
<td>103</td>
</tr>
<tr>
<td>Mean age at first pregnancy</td>
<td>19.95</td>
<td>18.76</td>
<td>1.39</td>
<td>0.16</td>
<td>84</td>
</tr>
<tr>
<td>Mean age at menarche</td>
<td>14.21</td>
<td>14.13</td>
<td>0.18</td>
<td>ns</td>
<td>87</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>4.41</td>
<td>10.79</td>
<td>-9.44</td>
<td>0.0001</td>
<td>100</td>
</tr>
<tr>
<td>Mean number of live births</td>
<td>3.77</td>
<td>10.07</td>
<td>-10.62</td>
<td>0.0001</td>
<td>106</td>
</tr>
</tbody>
</table>
A multiple regression analysis of the reproductive variables is performed with the aim of predicting completed family size in the Limon setting. The purpose of this analysis is to obtain an equation with the fewest number of independent variables, which predicts achieved fertility. Since twenty observations had missing values, this analysis is based on 88 subjects.

The best model obtained is:

\[ Y = 0.1794 X_0 - 1.09 X_1 + 0.80 X_2 + 0.04 X_3 \]

where

- \( Y \) = the predicted completed family size
- \( X_0 \) = a constant
- \( X_1 \) = the number of abortions
- \( X_2 \) = the total number of pregnancies
- \( X_3 \) = the age at menarche

The model incorporates the age at menarche, which marks the beginning of the reproductive career, and the total number of pregnancies and abortions, which approximate the number of live births. Table 27 provides the analysis of variance of the multiple regression model. This model maximizes the \( R^2 \) to 0.8551, and after adjustment for degrees of freedom, the \( R^2 \) is 0.8499. The model also minimizes the Mean Square error and the root MS error to 2.15 and 1.46 respectively. Both statistics indicate that the variance about the regression line is minimized in this model.
Table 27. Analysis of Variance of the multiple regression model

| Source of Variation | df | SS       | MS        | F         | P< 
|---------------------|----|----------|-----------|-----------|--------
| Model               | 3  | 1068.41690 | 356.13897 | 165.246   | 0.0001 |
| Error               | 84 | 181.03764  | 2.15521   |           |        |
| Corrected Total     | 87 | 1249.4555  |           |           |        |

Root MS error = 1.4680 \( R^2 = 0.8551 \)
\( R^2 \) adjusted = 0.8499

According to Draper and Smith (1981) the F ratio of the regression to the residual mean square must be at least 4 times the usual percentage point for the model to be practically useful, as opposed to only statistically significant. Following Draper and Smith's suggestion, for the degrees of freedom of the present model, the F ratio should be at least 7 times the usual percentage point (2.45) to yield a ratio of 17.15. The F ratio obtained in the present analysis, 165.246 clearly exceeds that guideline. Thus, this model is practically useful as opposed to a solely statistically-significant model.

The plot of the residuals against the predicted values is shown in Figure 5. The plot does not indicate that the regression assumptions have been violated. The plots of residuals (not shown) against the dependent and independent variables do not suggest any deviations.
Figure 5. A plot of the residuals against the predicted values.
A principal component (PC) analysis of the fertility data is performed with the aim of reducing the information of the raw data into a few uncorrelated indices. Thus, the variation of the original variables is condensed into a smaller number of indices, which affords a simpler interpretation of the fertility patterns in the Limon sample. Twenty-eight observations had missing values for various variables. Thus, the analysis is performed on data from only 80 subjects. Only the first 3 components are interpreted, thus explaining 76% of the variance. The meaning of the fourth component (12% of the variance) is not clear. Tables 28 and 29 summarise the results of the principal component analysis of the reproductive variables.

Table 28. Principle component (PC) analysis of the reproductive variables.

<table>
<thead>
<tr>
<th>Eigenvalue</th>
<th>Proportion</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1 2.94406</td>
<td>0.42058</td>
<td>0.42058</td>
</tr>
<tr>
<td>PC2 1.53046</td>
<td>0.218637</td>
<td>0.63922</td>
</tr>
<tr>
<td>PC3 0.89066</td>
<td>0.127238</td>
<td>0.76646</td>
</tr>
<tr>
<td>PC4 0.84126</td>
<td>0.120180</td>
<td>0.88664</td>
</tr>
<tr>
<td>PC5 0.67482</td>
<td>0.096402</td>
<td>0.98304</td>
</tr>
<tr>
<td>PC6 0.11724</td>
<td>0.016749</td>
<td>0.99979</td>
</tr>
<tr>
<td>PC7 0.00149</td>
<td>0.000214</td>
<td>1.00000</td>
</tr>
</tbody>
</table>
Table 29. Eigenvector analysis

<table>
<thead>
<tr>
<th>Reproductive variables</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed family size</td>
<td>0.531918</td>
<td>0.202300</td>
<td>-0.032384</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>0.052928</td>
<td>0.587878</td>
<td>0.443178</td>
</tr>
<tr>
<td>Multiple births</td>
<td>-0.002789</td>
<td>0.504622</td>
<td>0.593603</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>-0.120168</td>
<td>0.539494</td>
<td>-0.279817</td>
</tr>
<tr>
<td>First pregnancy</td>
<td>-0.258521</td>
<td>0.203290</td>
<td>0.589752</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>0.562107</td>
<td>-0.071207</td>
<td>0.143030</td>
</tr>
<tr>
<td>Number of live births</td>
<td>0.563042</td>
<td>0.146217</td>
<td>0.060145</td>
</tr>
</tbody>
</table>

The first component is a contrast between variables that maximize reproduction (live births, number of pregnancies and completed family size) and the age at menarche, which marks the beginning of the reproductive period. Figure 6 is a plot of the subjects along the second and first principal components. Women who have a greater than average number of pregnancies are represented by a '0', and those who have a lower than average number of pregnancies by a '*'. Subjects above the average are plotted on the positive side of the first component, and those below the mean on the negative side. The first component contrasts clearly women with high and low fertility.
Figure 6. A plot of the second and first PC's. Women with high fertility are represented by a '0', women with low fertility by a '*').
The second PC is interpreted as a dichotomy between two variables that have a positive impact on achieved fertility, and one variable that has a negative impact on achieved fertility. The positively-signed variables are menarche, which marks the beginning of the reproductive years, and multiple births, which is moderately correlated with the number of live births \((r=0.18, p<0.05)\). The negatively signed variable is the number of abortions, which has a negative impact on live births. A plot of the women along the third and second principal components is shown in Figure 7. The subjects are represented by a '0' if they have multiple births, and by a '*' if they do not. Although the plot does not produce two completely homogeneous clusters of numbers, both groups are differentiated.
Figure 7. A plot of the third and second PC's. Women with more than average multiple births are represented by a '0' (Group 2), women with less than average multiple births by a '*' (Group 1).
The third principal component is a dimension that contrasts the beginning of the reproductive years with actual childbearing. Thus, the age at menarche is negatively signed, and the age at first pregnancy and the number of multiple births is positively signed. The plot of the subjects along the first and third PC's does not yield a clear pattern, although the women who experienced late menarche tend to form a cluster in the negative side of the third component.
Figure 8. A plot of the first and third PC's. Women with late menarche are represented by a '0' (Group 2), women with early menarche by a '*' (Group 1).
CHAPTER FIVE
DISCUSSION

This dissertation examines the effects of hemoglobin S on the fertility of a sample of women in the Limon province of Costa Rica. The principal aim is to test the hypothesis of differential reproduction of Hb AA and Hb AS females in a malarial environment. Therefore, this study investigates the action of natural selection on human fertility when the selective agent is malaria. The primary biocultural reproductive components of achieved fertility are also studied. This dissertation does not demonstrate natural selection through differential fertility when the selective agent is malaria. However, differential reproduction is documented in a natural-fertility population as a result of various maternal characteristics.

The differential fertility hypothesis is difficult to test because it requires the screening of a large number of females, which is a costly process. For example, if the heterozygote frequency in a population is 1/10, then 1000 people must be sampled to obtain 100 heterozygotes. However, the sampling procedure must be restricted to post-reproductive females, because the hypothesis is tested by comparing the achieved reproduction of Hb AA and Hb AS females. Thus, the population must be large, and must have been exposed to malaria in an endemic fashion.

Since it is so difficult to obtain an appropriate number of subjects, Firschein (1961), and Custodio and Huntsman (1984), included in their samples women in their 20's and 30's, whose
complete fertility can not be measured. The oldest age category in Firschein's and Custodio and Huntsman's samples is 40+ years of age, without any specification on how many females are included who are 50 years and older, the age at which the rate of childbearing approaches 0.

Firschein's sample is larger than Custodio and Huntsman's and the present study's. However, Firschein's subjects were not selected randomly, but at a gynecological clinic, thus favoring "mothers". By contrast, the samples of this dissertation and of Custodio and Huntsman's study were randomly selected. Although the sample size of this dissertation is smaller than Firschein's, it has the advantage of including females above 40 years of age only. The present study's sample also allows the comparison of the reproductive performance of Hb AA and Hb AS subjects 50 years and older, which was not attempted by the two previous studies.

A random subsample of 28 women was re-interviewed, in order to quantify the reliability of the variables investigated. It was determined that for all variables, the two different interviewers do not significantly affect the subjects' responses. All reproductive variables are highly reliable, but the reliability of the number of siblings is not different from 0. Thus, the latter was not analyzed.

This investigation finds no reproductive advantage of the heterozygous females. Hb AA and Hb AS women do not differ in the mean number of pregnancies, abortions, live births, multiple pregnancies and completed family size. Neither group exhibits
different ages at menarche, at first pregnancy and at menopause. A discriminant function analysis fails to separate Hb AA and Hb AS females based on multiple reproductive variables. Thus, both the univariate and multivariate analyses concur.

This study contradicts the results of Firschein (1961, 1984) in his study of the Central American Black Caribs. However, this dissertation confirms prior reports by Custodio and Huntsman (1984) in Central America, and by Hamilton and collaborators (1972) in Africa, who found no reproductive advantage of Hb AS females under comparable circumstances. The absence of natural selection through fertility when the selective agent is malaria had been suggested previously by the studies of Allison (1956), Delbrouk (1958), Edington (1955), Garlick (1960) and Roberts and Boyo (1960). It is not clear why Firschein's results differ from those of previous and subsequent researchers. It is unlikely that Firschein documented an evolutionary process such as differential fertility that has not been demonstrated in similar studies. Given the evidence obtained from other reports and the present dissertation, it is more probable that an exogenous variable such as inadequate laboratory techniques, faulty sampling, or flawed fertility data account for Firschein's differing results. The mean number of live births and surviving children obtained by Custodio and Hunstman (1984) and by the present study are similar. In contrast, Firschein's mean values are consistently lower for Hb AA and higher for Hb AS females than those of the other two studies. Malaria control is not likely to account for the difference between Firschein's results and those of the other two studies. The investigations
that tested the differential fertility hypothesis during the 1950's and early 1960's took place in endemic malarial areas, but their results contradict Firschein's findings (Allison, 1956; Delbrouk, 1958; Edington, 1955; Garlick, 1960; Roberts and Boyo, 1960).

The conclusions of this investigation also agree with reports from the obstetrical and gynecological literature, which indicate that the effects of malaria on pregnant immune subjects are not an important evolutionary force. In immune subjects, malarial parasitemia and its clinical manifestations are exacerbated during pregnancy primarily among young primigravids. Particularly, the placenta is a privileged site for parasitic growth (Bray and Sinder, 1979; Brabin, 1983; Bruce-Chwatt, 1952; Jelly, 1969; McGregor, 1984; McGregor et al., 1983; Meerstadt, 1986; Playfair, 1982; Taufa, 1978; Vinayak et al., 1986; Williams and Mc Farlane, 1970). Pathological placental modifications attributed to *P. falciparum* decrease placental efficiency by reducing oxygen diffusion (Galbraith et al., 1980; Loke, 1982; Meerstadt, 1986; Strang et al., 1984). However, the effects of placental malaria on fertility are not clearly understood. Some studies suggest that young primigravids have an increased risk of fetal wastage as a result of malaria. Most studies indicate that the only deleterious effect of malaria on primigravids is low birth weight (Anagnos et al., 1986; Brabin, 1983; Gilles et al., 169; Loke, 1982; McGregor, 1984; McGregor et al., 1983; Strang et al., 1984). Therefore, although malaria affects the placenta, it is unlikely that the infection causes fetal wastage, the mechanism proposed by Firschein
to explain differential fertility in a malarial environment.

The path analyses of the fertility data do not indicate that Hb AA and Hb AS females differ reproductively. Rather, the path diagram of Hb AS and all subjects resemble each other more closely than they resemble the diagram of homozygous A females. The primary difference between the path analytical models of carriers and all subjects on one hand, and of homozygotes A on the other, concerns the correlation between multiple births and completed family size. These results are most likely caused by random sampling of multiple births between both subsamples.

Most path analyses of human fertility have not utilized as many reproductive variables as in this study. Path analysis of human fertility has concentrated on the elucidation of causal systems where the dependent variable is completed fertility or national fertility rates. The first line of research is macro studies of international fertility trends. The second is concerned with causal relations among various socioeconomic factors and reproductive variables in specific groups.

Macro studies on fertility rely on census data from different countries. (Balakrishnan et al., 1980; Chang et al., 1979; Choi, 1980; Ketema, 1985; Menard, 1973; Pathak and Murty, 1982; Rao, 1985; Roy, 1981; Talwalker, 1981; Tolnay and Christenson, 1984). These studies usually incorporate in the analysis variables such as per capita GNP, urbanization, literacy rate, infant mortality, contraceptive programs (Tolnay and Christenson, 1984), life expectancy, medical system, percentage of economically active women (Pathak and Murty, 1982), use of mass media, and diet and
nutrition (Chang et al., 1979; Menard, 1973; Rao, 1985). The strength of macro studies of fertility incorporating path analytical models lies in their evaluation of indirect effects on fertility (Asher, 1976). For example, these studies elucidate the indirect ways in which modernization affects fertility, e.g., education and medical services. Path analytical studies, then, render a more comprehensive view of international fertility trends. (Balakrishnan et al., 1980; Chang et al., 1979; Choi, 1980; Menard, 1973; Pathak and Murty, 1982; Rao, 1985; Roy, 1981; Talkalker, 1981; Tolnay and Christenson, 1984).

The second kind of studies on human fertility which incorporate path analytical models concentrate on specific communities. Thus, the research of this dissertation falls into the latter category. The main concern of these studies is to elucidate the input of socio-cultural and various reproductive variables on fertility (Ashmed, 1981; Costa, 1982; Chang, 1981; Chi and Harris, 1979; Gatara, 1982; Hodge and Ogawa, 1981; Ketema, 1985; Loebner and Driver, 1973; Nassirpour, 1984; Phananiramai, 1981; Roy, 1981; Shin et al., 1981; Simonen, 1981). The variables investigated are study-specific. Chi and Harris (1979), incorporate in their system only two biological variables (i.e., mother’s age, and previous child mortality), but utilize eight socio-economic variables. Other studies concentrate only on sociological variables (Ahmed, 1981; Chang, 1981; Gatara, 1982; Hodge and Ogawa, 1981; Ketema, 1985; Loebner and Driver, 1973; Nassirpour, 1984; Phananiramai, 1981; Roy, 1981), while still others (Costa, 1982; Shin et al., 1981;
Simonen, 1981) incorporate more reproductive aspects of the mother. This dissertation includes a total of 7 reproductive variables, although the final model has only 4.

In Limon, the three path analyses yield models that incorporate the number of multiple births, abortions and pregnancies as the independent variables, and completed family size as the dependent variable. These three models explain 86% of the variance. This analysis indicates that up to 86% of the variance of completed fertility may be explained by maternal reproductive variables. If these variables are genetically determined, natural selection may operate through differential reproduction in a natural fertility population. Completed family size may also be affected by environmental, socioeconomic, cultural and the like factors. Those variables are probably contained in the residual.

The analysis of the reproductive data yields several insights into the biocultural reproductive determinants of achieved fertility in the Limon sample. In the Limon sample, age at menarche is significantly correlated with the number of abortions ($r=-0.28, p<0.007$). These results confirm previous reports by Bracken et al. (1985), Leistol (1980), and Martin et al. (1983), which indicate that early menarche increases the risk of fetal wastage. However, these studies do not propose a mechanism to account for this correlation. Montagu (1979) suggests that the period of adolescent sterility results from the immature tract.

The Limon data set also confirms prior suggestions by Gray (1977) that women with early menarche may initiate their reproductive careers earlier. In Limon, women with early menarche
have a mean of 7.07 pregnancies, while those with late first pregnancy have a mean of 6.48 pregnancies, but the difference between both means is not significant.

When the data set was divided into women with completed family size below and above average, it was determined that the groups are significantly different only in their mean number of live births and pregnancies. Women with above average fertility do not start reproducing earlier than do their peers. The two main determinants of above average achieved fertility are the number of pregnancies and the number of live births. A multiple regression analysis of the fertility data yields an equation for the prediction of completed family size. The equation incorporates the number of abortions, pregnancies, and the age at menarche as the predictors. The principal component (PC) analysis of the reproductive data adequately condensed the information provided by the fertility variables. The first PC contrasts women with low and high fertility. The other PC's stress the importance of multiple births, the length of the reproductive period, the number of abortions and the length of time between menarche and first pregnancy. In sum, several biocultural reproductive variables influence achieved reproduction in the Limon sample. The number of pregnancies and of live births account for a greater than average completed family size. However, the achieved fertility for all subjects can best be predicted by their number of pregnancies and abortions and by their age at menarche. The principal component analysis combines the main reproductive variables that account for
fertility differentials among the subjects into 3 indices.
CHAPTER SIX

CONCLUSIONS

The primary conclusions of this dissertation may be summarized as follows:

1) There is no difference in the achieved fertility of homozygous AA and heterozygous AS females. This investigation fails to demonstrate any reproductive advantage of Hb AS women as proposed by Firschein (1961). The data collected in Limon indicate that the Hb S polymorphism is not maintained by differential fertility.

2) All reproductive variables are statistically reliable. The reliability of the subjects' answers was quantified with an intra class correlation coefficient (R). R was significantly different from 0 for all variables. However, the number of siblings has a low reliability estimate, probably as a result of the Limon family structure. Among black Limonenses, male-female unions tend to be unstable. Thus, the subjects in this study may have siblings who share the same parents, or just the same mother or father. The lack of concordance about how many siblings the women have, may be a result of the subjects' enumeration of all siblings in one interview, and some of them in the other. The reliability estimate for this variable is not significantly different from 0.

3) Women who experience early menarche have a greater risk of fetal wastage. The mechanism accounting for this risk is not understood.

4) In the Limon sample, the best multiple regression equation
for the prediction of completed family size (CFS) is the following:

\[ CFS = 0.1794(a) - 1.09(X_1) + 0.80(X_2) + 0.04(X_3) \]

where

- \( a \) = a constant
- \( X_1 \) = number of abortions
- \( X_2 \) = number of pregnancies
- \( X_3 \) = age at menarche.

6) Achieved reproduction in the Limon sample is influenced by varying ages at menarche, and from different numbers of pregnancies, multiple births, abortions and live births.

In conclusion, this dissertation fails to demonstrate any reproductive advantage of heterozygous females for the Hb S gene. However, this study documents general differential reproduction in a natural fertility population. In the Limon sample, up to 86% of the variance of completed family size can be explained by maternal characteristics such as the length of the reproductive period, the number of pregnancies, of abortions and multiple births. If these traits are determined genetically, natural selection may act through differential fertility favoring women with greater than the average completed family sizes. Mac Cluer (1978) indicates that the potential for the action of natural selection through differential reproduction in humans is important. The response of fitness to natural selection has been estimated by R. A. Fisher in his fundamental theorem. The theorem states that "the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at any time" (Fisher, 1958). Fisher
notes that even under the action of natural selection, fitness does not necessarily increase with time. This is a consequence of the environment changing constantly, and the standard of fitness thus changing continuously (Edwards, 1967; Falconer, 1985; Haldane, 1949; Kimura, 1958; Penrose, 1949; Price, 1972). Thus, Fisher's fundamental theorem provides an evolutionary frame of reference for studies of differential reproduction in natural fertility populations. Fisher contends that these differences in fertility may not necessarily result in evolutionary change because the forces which cause differential reproduction change constantly. Therefore, the fitness of all individuals in a population never equals 1.

In conclusion, this dissertation documents differential fitness in a natural fertility population. However, as R A Fisher (1958) has noted, such differential reproduction may not necessarily result in evolutionary change.
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N.___
1- Age____________________
2- Name___________________________________________________________
3- Date of birth___________
4- Place of birth_______________________________________________________
5- How many brothers and sisters do you have?
   ______alive brothers, _______alive sisters
6- How many children do you have?
   ______living sons, _______living daughters
   ______dead sons, _______dead daughters.
7- How old were you, when you had your first menstrual period?
   ________________________________

8- How old were you when you had your FIRST PREGNANCY?___________
   8.1- Was the baby born?   Yes   No
   8.2- If yes:   Female   Male
           Twins MF MM PF MZ DZ
9- How long did you breast feed him/her?_______________________________
   ________________________________
10- Is the child alive?   Yes   No
   If the child died:
   11- Child's age.____________________________________________________
   12- What was the cause of death?____________________________________
   If the baby was not born:
   12- How advanced was the pregnancy?________________________________
   14- Were you sick when you had the miscarriage______________________
15- How old were you when you had your SECOND PREGNANCY?

15.1- Was the baby born? Yes No

15.2- If yes: Female Male
 Twins MF MM FF MZ DZ

16- How long did you breast feed him/her?

17- Is the child alive? Yes No

If the child died:

18- Child's age.

19- What was the cause of death?

If the baby was not born:

20- How advanced was the pregnancy?

21- Were you sick when you had the miscarriage