

## A long-term study of non-native-heartworm transmission among coyotes in a Mediterranean ecosystem

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In Mediterranean ecosystems, abiotic factors are known to affect vertebrate population dynamics, but little is known about how these factors affect population dynamics of parasites. We conducted a 9-year investigation of the roles of temperature, precipitation, and vector abundance as determinants of transmission of the non-native canine heartworm (*Dirofilaria immitis*), a dangerous parasite of pets, among coyotes (*Canis latrans*), an important reservoir, in north-coastal California. Dates of heartworm transmission and total annual transmission were determined, respectively, from lengths and numbers of heartworms found in known-age coyotes. Vector host-seeking activity was assessed through weekly mosquito trapping. Within years, heartworm transmission occurred only when cumulative temperatures were sufficient to allow larval heartworms to develop to the infective stage (as predicted by an existing degree-day model), and when suitable vectors were available. Most (95%) heartworms infected their hosts between 1 July and 14 September. The onset of transmission periods always occurred after the peak in vector host-seeking activity and varied annually. Transmission periods ended before temperatures became limiting due to absence of vectors. The timing of host-seeking activity of the primary vector species, *Ochlerotatus sierrensis*, also was correlated with the onset of warming temperatures such that parasite and vector phenology were synchronized. For this reason (partly), the variation in timing of seasonal warming had no detectable effect on total annual transmission. Abundance of host-seeking *Oc. sierrensis* was positively correlated with annual precipitation, and annual heartworm transmission was positively correlated with abundance of host-seeking *Oc. sierrensis*. Annual transmission also was positively correlated with abundance of a less numerous vector species, *Anopheles punctipennis*, and was directly correlated with precipitation. This study demonstrates that multiannual variability in temperature, which affects seasonality of transmission, has little effect on annual transmission, but that precipitation is a driving force determining annual transmission. These findings imply that in California, and possibly other Mediterranean climate zones, it is especially important to preventively treat pets in summers following high-rainfall winters.

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In Mediterranean climate zones, which are characterized by hot, dry summers and cool, wet winters, abiotic factors such as temperature and precipitation can be important determinants of vertebrate population dynamics. For example, winter precipitation is known to

drive many herbivore populations through bottom-up trophic processes (Garsd and Howard, 1981, Fuentes and Campusano 1985, Jimenez et al. 1992, Heske et al. 1997). Precipitation and temperature also may play fundamental roles in determining population dynamics

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of vector-borne parasites in Mediterranean ecosystems through effects on transmission (i.e. parasite recruitment). Although it is well established that these and other abiotic environmental factors affect geographic patterns of parasite abundance (Hess et al. 2002, Sacks 2002), little is known about how multiannual variability in these factors affect transmission over time.

The present study explored the effects of temperature, precipitation, and vector abundance on transmission of canine heartworm (*Dirofilaria immitis*) among coyotes (*Canis latrans*) in northern California. Heartworm is a geographically invasive filarial nematode parasite of significance primarily to domestic and wild canids and, to a lesser extent, humans and other mammals (Otto 1975, Sacks and Blejwas 2000). This parasite has expanded its geographic range considerably worldwide over the past century, including the recent spread westward in North America to California (Lok 1988, Sacks and Caswell-Chen 2003). Coyotes represent an important reservoir maintaining this parasite in the environment (Sacks 1998, Sacks and Blejwas 2000). An impetus for this study was to aid in the development of predictive models associated with this economically and ecologically important parasite.

Although temperature affects host-seeking activity and survival of mosquitoes (Bennett 1980, Otto and Jachowski 1981), the primary effect of temperature on transmission likely relates to constraints on development of larval heartworms. Heartworm has an indirect lifecycle, requiring a canid definitive host, in which it sexually reproduces, and a mosquito intermediate host, in which it undergoes two larval molts. Female mosquitoes become infected with microfilariae when they blood-feed on an infected canid host, and infected mosquitoes are vectors of third-stage heartworm larvae when they feed on subsequent canid hosts (Abraham 1988). Even if host-seeking vectors are abundant, transmission cannot occur unless cumulative temperatures are sufficiently high for larval heartworms to develop to the infective (L3) stage (Fortin and Slocombe 1981). This requirement for transmission is often quantified using the "heartworm development unit" (HDU) model (Slocombe et al. 1990), which is a degree-day model based on a 30-day running sum of average daily temperatures (see Material and methods for details). Thus, one potential effect of temperature is to limit transmission to certain periods of the year (Knight and Lok 1996), which vary in seasonal timing from one year to the next.

The annual timing of such periods also may affect total annual transmission because the seasonality of host-seeking vector activity depends partly on factors independent of temperature (e.g. photoperiod; Garcia and Ponting 1972, Ahmadi et al. 1985), potentially resulting in variable overlap between the two factors. In northern California, *Ochlerotatus sierrensis*, the western treehole mosquito, is thought to be the primary heart-

worm vector (Weinmann and Garcia 1974). Emergence of adult female *Oc. sierrensis* in this region usually peaks from mid April to mid May (Woodward et al. 1988, Garcia et al. 1989, Washburn et al. 1989), well before temperatures warm to levels predicted to allow larval development of *D. immitis* in the vector (Knight and Lok 1996). Emergence is >90% complete by the end of June. The timing of pupation and, therefore, emergence of adult mosquitoes is relatively constant among years due to the close dependence of this process on day length. In contrast, the time of year at which ambient temperatures become high enough for heartworms to develop in vectors – although generally later than peak *Oc. sierrensis* emergence – varies among years, such that the seasonal timing of increased temperatures could be an important factor determining heartworm transmission in any given year. That is, transmission may be higher in early-warming years if this means that higher numbers of vectors are present when temperatures are conducive to transmission. Alternatively, adult mosquito host-seeking activity may be delayed after emergence until temperatures warm to a certain point (Lee 1971, Bennett 1980), which could serve to synchronize mosquito host-seeking activity with periods conducive to rapid larval heartworm development. It remains an empirical question whether multiannual variability in the timing of warming summer temperatures affects total annual transmission of heartworm or whether it leads only to variability in the seasonal timing of transmission.

Other potential vectors of canine heartworm in north-coastal California, including *Culiseta inornata*, *Cs. incidens*, *Anopheles freeborni*, *An. punctipennis*, and *Culex tarsalis*, emerge later than *Oc. sierrensis* and peak in abundance after summer temperatures have warmed sufficiently to enable larval development of heartworms. Based on laboratory studies, *An. freeborni* and *An. punctipennis* are highly competent vectors of *D. immitis* (Walters 1996). *Culiseta incidens* and, especially, *Cs. inornata* and *Cx. tarsalis* apparently have lower vector competence (Yen 1938, Walters 1996, Theis et al. 2000), but could still be important in transmission cycles if their numbers were sufficiently high relative to more efficient vectors (Theis et al. 2000). Thus, the importance of timing of warmer temperatures depends in part on the relative contribution as vectors of these late-emerging mosquito species versus the early-emerging *Oc. sierrensis*.

The most important effects of precipitation on transmission probably relate to vector abundance. Previous studies suggest that the annual abundance of the tree-hole breeding *Oc. sierrensis* should be positively related to annual (primarily winter) precipitation. For example, during drought years, smaller tree holes often dry out before larvae complete development (Garcia and Ponting 1972, Garcia et al. 1989), while larger tree holes retain smaller volumes of water, which can in-

crease larval mortality due to competition for food and space (Washburn et al. 1989, Colwell et al. 1995). Although all mosquito species require water to breed, population sizes of other potential heartworm vectors may be less directly related to precipitation (Vandyk and Rowley 1995). For example, *An. freeborni* in California breed primarily in rice fields and agricultural seepage, which depend more directly on human activity (e.g. irrigation) than on precipitation (Washino and Thomas 1985). Thus, as with temperature, the importance of precipitation on transmission is likely to depend on which vectors are most abundant.

## Hypotheses and approach

We tested predictions generated from three hypotheses about the relationships among temperature, precipitation, and heartworm transmission (Table 1). We based predictions regarding temperature limitation of larval heartworm development on the HDU degree-day model (Slocombe et al. 1990). Although this model has been validated for eastern North America (McTier et al. 1993, Lok and Knight 1999), localized strains of *D. immitis* in the west could have adapted to different climatic conditions (Otto 1975, Walters 1996). Investigation of hypothesis I, heartworm transmission periods in north-coastal California are constrained by temperature as predicted by the HDU model, required assessment of the seasonal period of transmission. We did this by testing predictions about the seasonal occurrence of immature or 5th stage larval (L5s) heartworms in the cardiopulmonary vasculature (McTier et al. 1993, Capelli et al. 1996) of wild-caught coyotes. To investigate the second hypothesis, late-warming years experience lower transmission than early-warming years (Table 1), we compared multiannual variation in host-

seeking seasons of *Oc. sierrensis* with seasons of temperatures putatively conducive to transmission (i.e. larval *D. immitis* development in the vector). We examined the relationship between annual transmission, i.e. the average number (adjusted for coyote age) of heartworms found in coyotes killed after that year's transmission season, and the date at which temperatures warmed sufficiently for transmission according to the HDU model. We surveyed the mosquito fauna to determine relative abundance and seasonal patterns of host-seeking activity by potential vector species. To investigate the third hypothesis, high-precipitation years support higher transmission than low-precipitation years, we examined multiannual relationships between precipitation and mosquito abundance and between these variables and heartworm transmission.

## Material and methods

### Study sites

Coyote carcasses were obtained from three counties in north-coastal California, Mendocino, Sonoma, and Napa, although most analyses used coyotes and mosquitoes from southeastern Mendocino County, California, USA. In Mendocino County, mosquitoes were trapped near Potter Valley (PV; 39°14'N, 123°06'W) during 1995–2001 and coyotes were trapped 20 km south at the 21-km<sup>2</sup> Hopland Research and Extension Center (HREC; 39°00'N, 123°05'W) during 1994–2002. The distance separating the two Mendocino County sites was small relative to the dispersal distance of coyotes ( $\leq 300$  km; Hawthorne 1971, Harrison 1992). The two sites also had similar seasonal and multiannual patterns of emergence and abundance of adult female mosquitoes (Lee 1971, Woodward et al. 1988, Garcia et al. 1989, Washburn et al. 1989), which

Table 1. Hypotheses and predictions regarding relationships between temperature and precipitation, and transmission of *D. immitis* in coyotes.

Hypothesis	Predictions
I. Transmission in north-coastal California is limited by temperature as predicted by the HDU model of Slocombe et al. (1990)	<ol style="list-style-type: none"> <li>(1) Occurrences of heartworms in first-year coyotes and of immature L5 heartworms in all coyotes are seasonal</li> <li>(2) Infection dates, estimated from the lengths of immature L5s, occur within the HDU-predicted period</li> <li>(3) Estimated infection dates do not coincide with <i>Oc. sierrensis</i> host-seeking outside the HDU-predicted period</li> </ol>
II. Late-warming years experience lower transmission than early-warming years in north-coastal California due to reduced temporal overlap between <i>Oc. sierrensis</i> host-seeking and temperatures conducive to transmission	<ol style="list-style-type: none"> <li>(1) <i>Ochlerotatus sierrensis</i> host-seeking periods are independent of temperature</li> <li>(2) Transmission is inversely correlated with first date when HDUs are conducive to transmission</li> <li>(3) Late-emerging mosquito species make a small contribution to the vector pool</li> </ol>
III. High-precipitation years support higher transmission than low-precipitation years in north-coastal California	<ol style="list-style-type: none"> <li>(1) Annual abundance of important vectors is positively correlated with precipitation</li> <li>(2) Annual transmission is positively correlated with annual abundance of vectors and, to a lesser extent, precipitation</li> </ol>

was confirmed during the study by mosquito sampling at HREC in one year (Sacks 2002). Temperature and precipitation data were collected from HREC weather stations (<http://www.ipm.ucdavis.edu/WEATHER/wxretrieve.html>).

### Data collection

Female host-seeking mosquitoes were trapped weekly from March to October of the seven years at the PV site using Fay traps (Fay and Prince 1970) baited with CO<sub>2</sub> as per Garcia et al. (1989) and Washburn et al. (1992) (hereafter, Fay/CO<sub>2</sub> traps). Dead mosquitoes were taken to the laboratory, identified to species according to Bohart and Washino (1978), and counted. Live mosquitoes were anesthetized with CO<sub>2</sub> in the field and released after identification and counting.

Coyote carcasses were obtained from USDA/APHIS/Wildlife Services specialists after coyotes had been trapped or shot during routine predator control activities (i.e. for reasons unrelated to our research). Based on a study of radio-collared coyotes at HREC, heartworm infection apparently had no effect on a coyote's probability of being trapped or shot (Sacks and Blejwas 2000). Therefore, our sample should have been unbiased with respect to heartworm prevalence and intensity. Coyotes were necropsied and examined for heartworms as previously described (Sacks 1998). All undamaged heartworms were sexed and measured (mm total length). Coyotes were aged via cementum annulus counts using a section of a lower canine or premolar root (Matsons Laboratory, Miltown, MT; Linhart and Knowlton 1967).

### Seasonality of transmission and the HDU season

We examined the monthly prevalence of heartworm in first-year coyotes from Napa, Sonoma, and Mendocino Counties to assess seasonality of transmission over a broad north-coastal California region. First-year coyotes were used to ensure that all heartworm infections observed were obtained in the most recent transmission season.

We then examined the lengths of heartworms found in coyotes at HREC to determine whether the occurrence of immature heartworms, i.e. 5th stage larvae (L5), was restricted to the fall. Heartworms typically require 70 days to reach the 5th stage and become established in the cardio-pulmonary vasculature, and reach adult size by 6 months (Kotani and Powers 1982). Therefore, with summer transmission, immature heartworms should have been observed in the fall. To minimize the confounding effect of small adults, we defined an "immature" heartworm as one being shorter than 3 SDs less than the average adult length reported by

Kotani and Powers (1982). This cut-off was expected to result in 1 incorrectly classified "immature" per 1,000 adult heartworms measured (Zar 1984).

We determined the start and end dates of seasonal temperatures conducive to larval heartworm development in the vector, and, hence, transmission, as predicted by the 30-day HDU model (Slocombe et al. 1990). We tested these predictions using measurements of immature heartworms to estimate dates of transmission. For each year, we calculated this hypothetical temperature-limited transmission period, referred to hereafter as the "HDU season." For each day, the heartworm development threshold (14°C) was subtracted from the average temperature for that day (estimated as half the sum of the maximum and minimum daily temperature) to produce a daily HDU. For example, on a day with temperatures ranging from 10 to 30°C, the daily HDU would be  $(10^{\circ}\text{C} + 30^{\circ}\text{C})/2 - 14^{\circ}\text{C} = 6^{\circ}\text{C}$ . Next, a 30-day HDU was calculated for each day as the sum of daily HDU for the current and previous 29 days. We adopted the conventional assumption, based on mosquito longevity, that most transmission occurs by mosquitoes that have acquired microfilariae within the most recent 30 days (Slocombe et al. 1990, McTier et al. 1993, Knight and Lok 1996). Thus, the HDU season each year was from the first day when the 30-day HDU exceeded 130°C to the last day when the 30-day HDU exceeded 130°C. We estimated the date of coyote infection of each immature heartworm according to a growth curve derived from Kotani and Powers (1982) and compared these infection dates to the HDU season. Because male heartworms are shorter than females, their growth curve is flatter than the female growth curve. Therefore, only lengths of immature females, which should have provided more accurate estimates of infection date, were used to infer dates of transmission.

### Does timing of HDU season affect annual transmission?

To test the prediction that host-seeking periods of *Oc. sierrensis* were independent of temperature, we calculated the Pearson correlation coefficient for the relationship between the first Julian date of the HDU season and the Julian date by which 95% of the *Oc. sierrensis* were caught. We chose to mark the "end" of the *Oc. sierrensis* host-seeking period based on 95% instead of 100% of the annual catch to reduce the sensitivity to chance captures of small numbers of late-season mosquitoes.

We tested the prediction that transmission was inversely correlated with the first Julian date of the HDU season. We defined annual transmission for each year as the average number of heartworms per coyote that

were acquired during that year's transmission season. To estimate annual transmission, all immature heartworms were assumed to have been acquired in the most recent transmission season. Otherwise, for coyotes killed before November of their second year, heartworms were assumed to have been acquired during the first transmission season of their life and for coyotes killed after October of their second year, the total number of heartworms was divided by the number of transmission seasons they had experienced (i.e. through November). The division of a coyote's heartworm burden into equal sized groups apportioned to each transmission season was conservative because it assumed that transmission was constant among years (which was the null hypothesis).

To assess the importance of potential vector species other than *Oc. sierrensis*, we examined capture numbers of host-seeking females of other species. Different species may have been differentially vulnerable to Fay/CO<sub>2</sub> traps, precluding precise quantification of relative abundance. However, most of the species identified in this study have been caught in large numbers using similar traps at other locations (D. L. Woodward, unpubl.). Thus, large differences in capture numbers between species were assumed to indicate gross differences in their relative abundance.

### Effects of precipitation on transmission

We calculated Pearson correlation coefficients for the relationships between annual precipitation (measured from 1 July of the previous year to 30 June of the current year) and annual numbers of each mosquito species. Then, we calculated Pearson correlation coefficients for the relationships between annual transmission of heartworm and annual numbers of each mosquito species. We performed a multiple regression analysis using as transmission predictors all mosquito species that had significant univariate correlations. To assess the relative strengths of pathways among precipitation, vector abundance, and transmission, we used standardized regression coefficients or  $\beta$  weights (Loehlin 1987).

## Results

### Seasonality of transmission and the HDU season

Based on 88 first-year coyotes killed September through March of 1994–2002 in the three north-coastal California counties, heartworms were absent from the cardiovascular vasculature until the end of October and prevalence reached a plateau by November (Fig. 1). From 61 coyotes (0–9 years old) killed at the HREC site in Mendocino County, we recovered and measured 1,291 heartworms (632 F, 659 M). The occurrence of

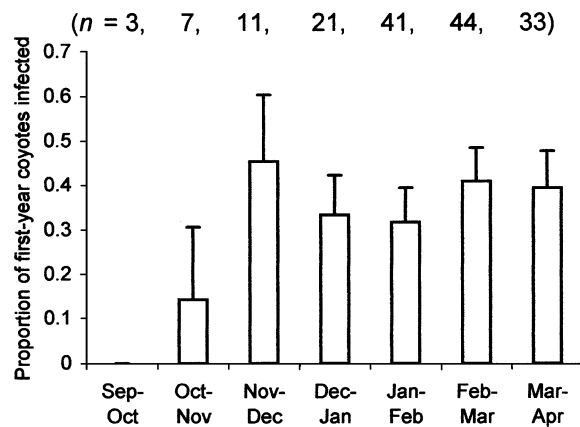


Fig. 1. Two-month running average of heartworm prevalence in 88 first-year coyotes from Mendocino, Sonoma, and Napa Counties, illustrating an increase from late summer through early fall and plateau by late fall, 1994–2002. The earliest observation of an infected coyote was 31 October.

immature heartworms ( $n = 112$ , 154, female, male, respectively) was seasonal, corresponding to late fall and early winter (Fig. 2). All coyotes infected before November were  $\geq 2$  years old.

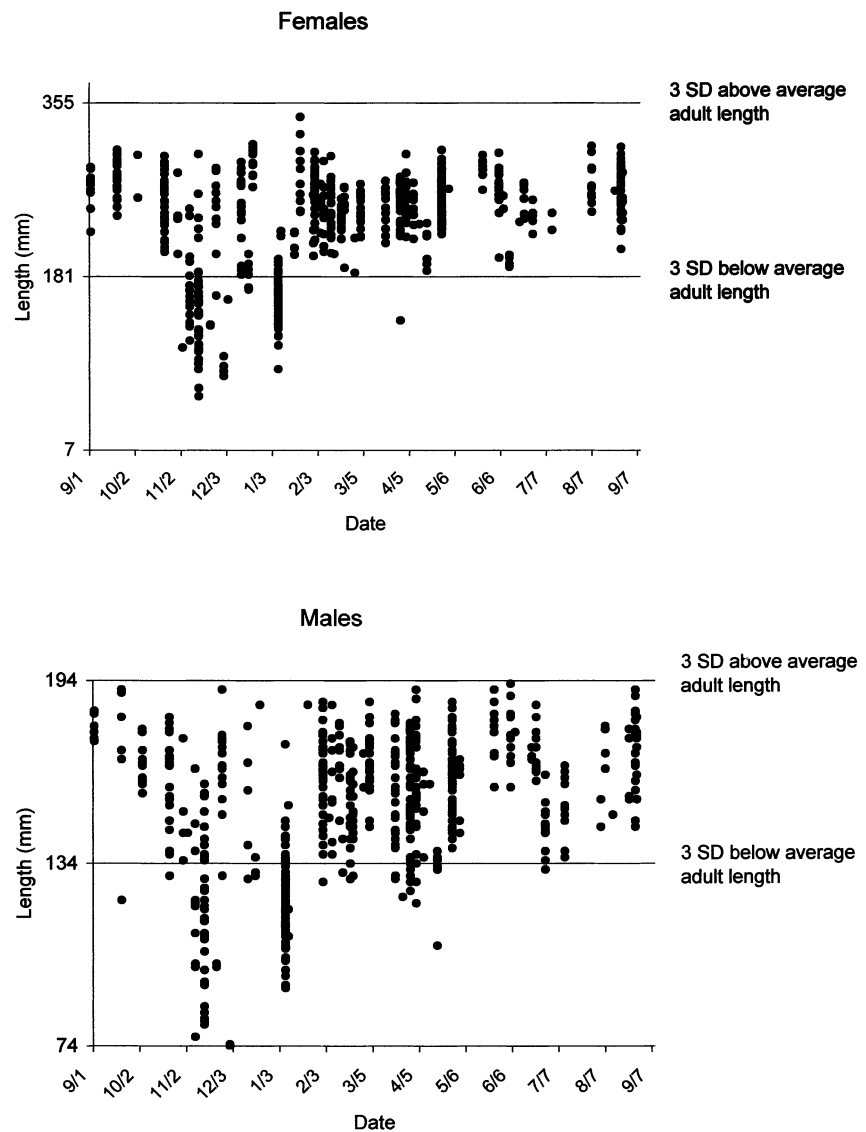
The timing of the HDU seasons varied among years, with start dates ranging from 21 May (2001) to 5 July (1995) and end dates ranging from 7 October (1998) to 31 October (2001). Most immature female heartworms were estimated to have infected coyotes between the latest start date (7/5) and the earliest end date (10/7) of the HDU seasons (Fig. 3A). Of six immature female heartworms estimated to have infected coyotes outside this period, we estimated five to have been transmitted between 6/30/97 and 7/2/97, well within the HDU season for that year (Fig. 3B) (5/27 to 10/16/97). The remaining female heartworm was estimated to have infected her coyote host after the last day of the HDU season (11/30/97).

Although heartworms were not transmitted to coyotes before the first day of the HDU season, female *Oc. sierrensis* were abundant well before this date (Fig. 4). In fact, during most years, peak abundance of host-seeking *Oc. sierrensis* occurred before the onset of the HDU season.

### Does timing of HDU season affect annual transmission?

There was a positive correlation between the onset of the HDU season and the end of the *Oc. sierrensis* host-seeking period ( $r = 0.85$ ;  $P = 0.015$ ), such that the lengths of the overlap between these periods were relatively constant among years (Fig. 5). Consistent with this synchrony between onset of the HDU season and end of the *Oc. sierrensis* host-seeking season, annual transmission (i.e. average number of new heartworms

Fig. 2. Lengths of 632 female and 659 male heartworms found in coyotes in relation to dates in Mendocino County, California, USA, 1994–2001. Heartworms shorter than 3 SD below average adult length ( $n = 112$  females, 154 males) were considered immature.



acquired per coyote) did not show a negative correlation with the onset of the HDU season (Fig. 5;  $r = 0.66$ ,  $P = 0.11$ ) as would have been expected if the two seasons were mutually independent.

Host-seeking periods of other potential vectors averaged and/or ended later in the year than that for *Oc. sierrensis* and of these, catches of all but two *Culiseta* spp. peaked during the HDU season in all years (Fig. 6). Although females of nine species were captured, two of those accounted for > 97% of the total number of mosquitoes caught.

### Effects of precipitation on transmission

Correlations between annual numbers of host-seeking mosquitoes and annual precipitation were significant

only for *Oc. sierrensis* ( $r = 0.94$ ,  $P = 0.002$ ) and *Cx. tarsalis* ( $r = 0.77$ ,  $P = 0.045$ ). Annual transmission of heartworm was closely correlated with annual numbers of female *Oc. sierrensis* ( $r = 0.90$ ,  $P = 0.006$ ) and *An. punctipennis* ( $r = 0.87$ ,  $P = 0.01$ ; Fig. 7), but was not significantly correlated with annual numbers of other mosquito species ( $P \geq 0.13$ ). A multiple regression model incorporating both *Oc. sierrensis* and *An. punctipennis* as predictors of annual transmission had greater explanatory power than univariate regressions with either species ( $R^2_{\text{adj}} = 0.86$  for both species, 0.76, 0.72 for each species, respectively). The two explanatory variables (*Oc. sierrensis*, *An. punctipennis*) were somewhat intercorrelated ( $r = 0.73$ ). Annual transmission also was directly correlated with annual precipitation during the seven years in which mosquitoes were sampled ( $r = 0.76$ ;  $P = 0.046$ ) and, with marginal signifi-

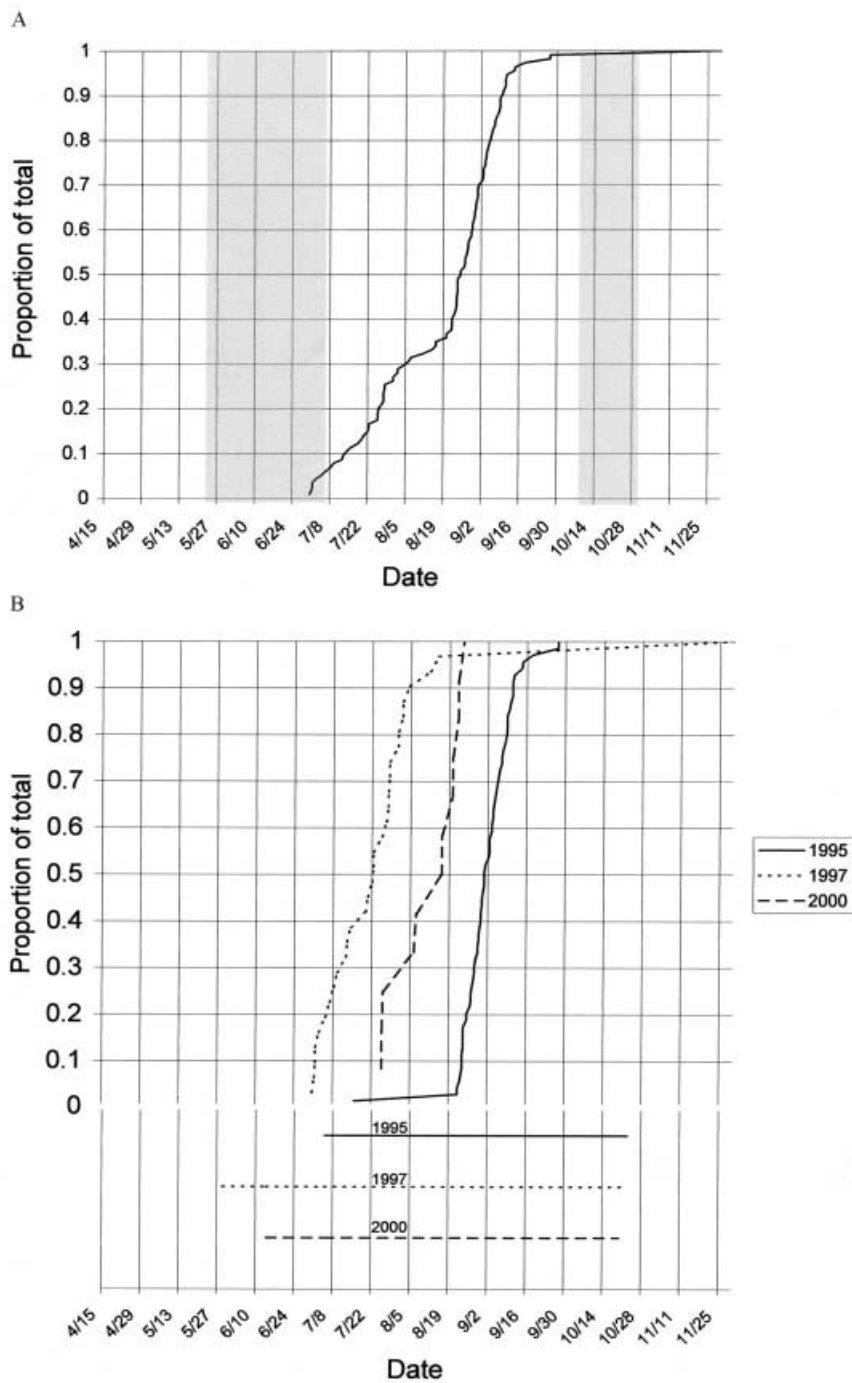


Fig. 3. Cumulative numbers of female heartworms recovered from coyotes in relation to estimated dates of infection in Mendocino County, California, USA: (A) pooled 1994–2001 (ranges of first days and last days of HDU seasons 1995–2001 are indicated in gray) and (B) shown separately for three years (HDU seasons are indicated separately for three years as horizontal lines and four years were not shown due to low sample size).

cance, during the full nine years of the study ( $r = 0.66$ ;  $P = 0.052$ ). The strength of the direct pathway from precipitation to transmission was greater than that of the indirect pathway from precipitation, via abundance of *Oc. sierrensis*, to transmission and similar to that of the combined indirect pathways involving both vector species (Fig. 8).

## Discussion

We investigated whether temperature and precipitation affected multiannual population dynamics of canine heartworm. Temperature was hypothesized to (1) limit the time period over which larval development was possible, and (2) do so variably from one year to the

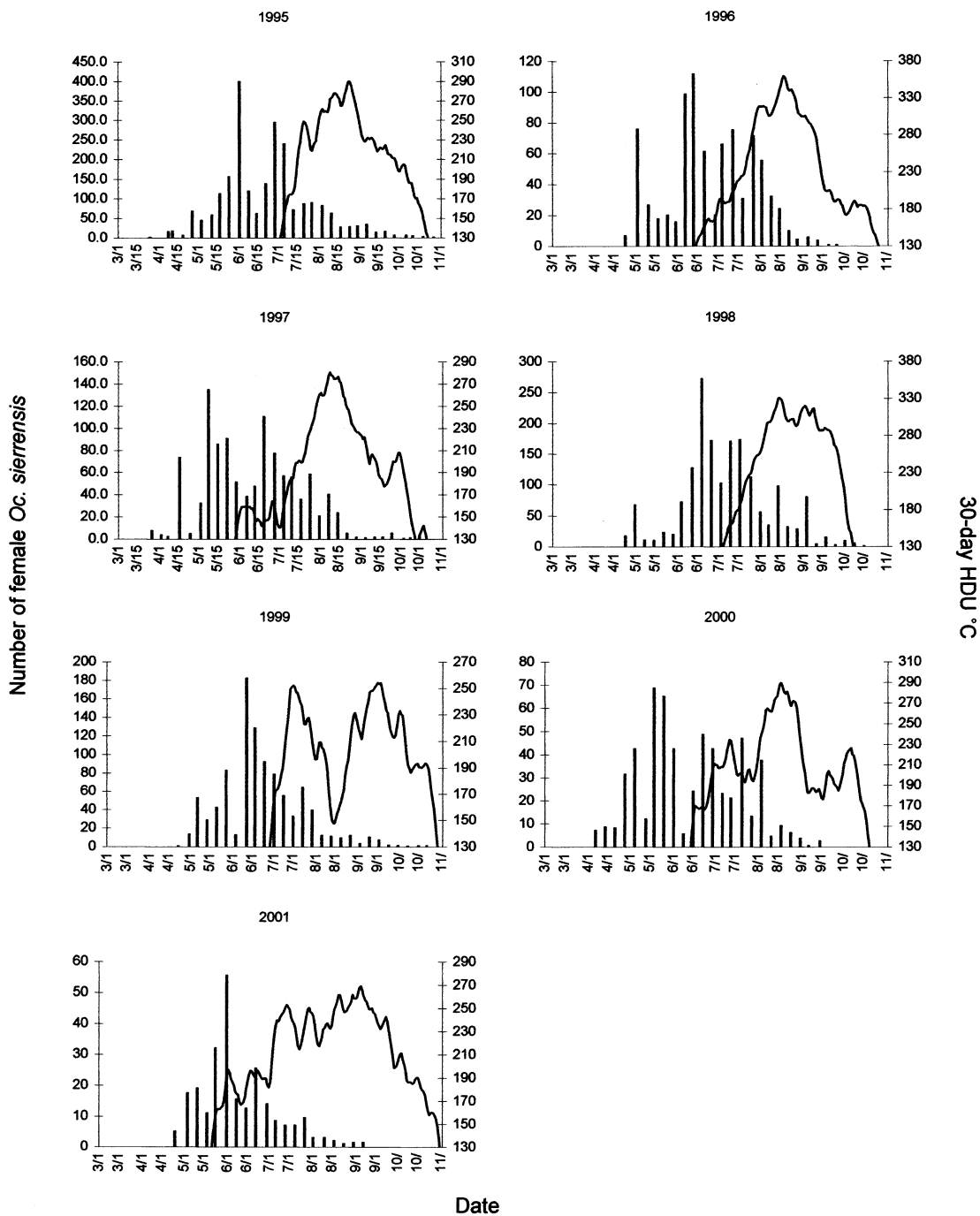


Fig. 4. Weekly catches of adult female *Ochlerotatus sierrensis* (bars) in two Fay/CO<sub>2</sub> traps at the PV site in relations to 30-day HDU (solid line), Mendocino County, California, USA, 1995–2001. Heartworms are expected to develop to the infective stage within vectors in  $\leq 30$  days only when the daily HDU for the previous 30 days sums to  $> 130^{\circ}\text{C}$ .

next, independently of the host-seeking season of vectors, such that transmission would be inversely related to the date of onset of the HDU season. Precipitation was hypothesized to directly influence abundance of important vectors. Our findings demonstrate the role of temperature in limiting heartworm transmission but

provide no evidence that the timing of warming temperatures affects the total amount of annual transmission. In contrast, precipitation did appear to influence the total amount of annual transmission via its effect on vector abundance. These findings are discussed in greater detail below.

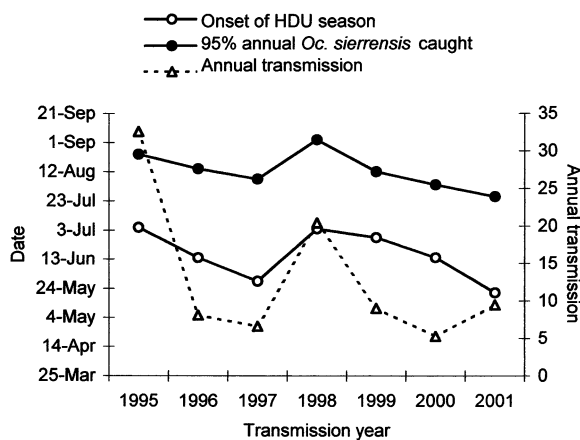


Fig. 5. Annual dates marking the first day of the HDU season and the day by which 95% of *Ochlerotatus sierrensis* were caught in relation to annual transmission (average annual numbers of heartworms infecting coyotes), Mendocino County, California, USA.

That temperature limited heartworm transmission to a narrower season than if vector abundance was the only transmission determinant was supported by the pattern of monthly prevalence in first-year coyotes from the three-county region, the seasonal pattern of immature heartworms in coyotes from HREC, and the estimates of transmission dates based on the immature heartworms. If vector abundance had been the only factor determining transmission, the latter would have been frequent before the onset of the HDU season, when transmission was never observed. We only estimated transmission to occur after the HDU season once, based on a single female heartworm. Given that we measured > 500 adult female heartworms, we stood an approximately 40% chance of misclassifying  $\geq 1$  of these as immature (i.e. 3 SD under the average adult length). Thus, our results agree with predictions of the HDU model. Although previous studies have demonstrated the accuracy of the HDU model for predicting transmission limits in eastern North America (McTier et al. 1993, Lok and Knight 1999), our results are the first to do so in western North America.

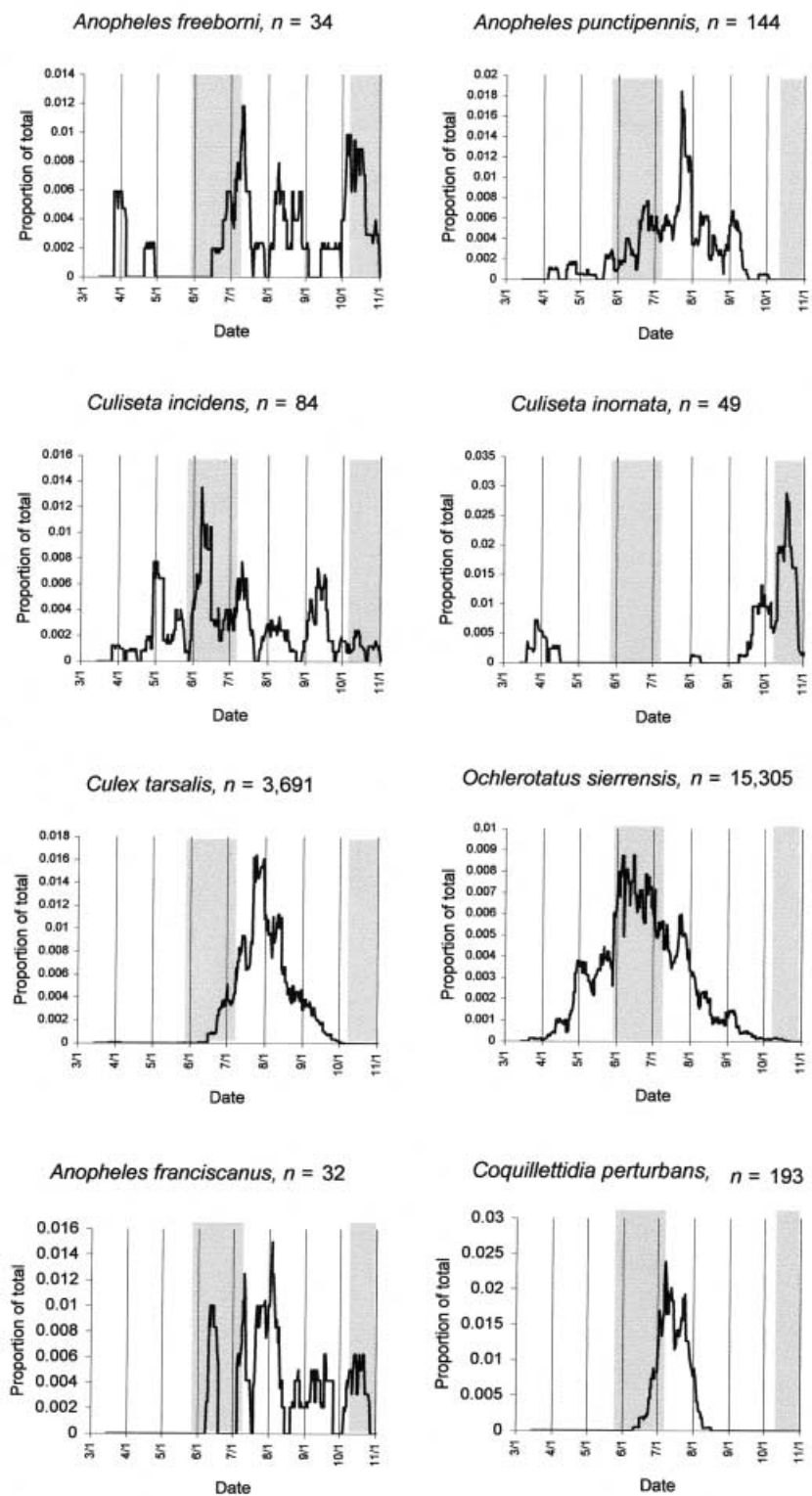
Despite the importance of temperature as a limiting factor of heartworm transmission, multiannual variability in the onset of the HDU season had no apparent effect on total annual transmission. Although the timing of female *Oc. sierrensis* emergence was likely to be relatively constant among years (Garcia and Ponting 1972), the host-seeking activity of adult females was probably delayed until temperatures warmed to a certain point (Lee 1971, Bennett 1980), explaining the synchrony observed in this study between the onset of the HDU season and the end of the *Oc. sierrensis* host-seeking season. That we found no inverse relation-

ship between onset of the HDU season and annual transmission implies also that *Oc. sierrensis* mortality was especially low while resting during the delay between emergence and host-seeking. If resting mortality had been similar to that associated with host-seeking there should have been a negative relationship between onset of the HDU season and transmission, despite the delay in host-seeking activity, due to continuous attrition of mosquito abundance.

While the variability among years in the onset of the HDU season did not affect total annual transmission, it did affect the seasonal timing of transmission. Among the three years from which we had reasonable sample sizes of immature heartworms, the onsets of the HDU seasons occurred in the same sequence (i.e. earliest to latest) as the median (for example) dates of transmission for these years (Fig. 3B). Other aspects of temperature (i.e. besides seasonal timing), not examined in this study, may affect heartworm population dynamics (Sacks 2002). For example, higher total annual HDU might lead to increased efficiency of transmission due to faster development of heartworm larvae. On the other hand, such efficiency could be off-set by reduced host-seeking activity (Lee 1971) and increased mortality (Otto and Jachowski 1981) of vectors at the higher temperatures. These relationships between temperature and heartworm transmission efficiency require further study.

In contrast to temperature, precipitation was a driving force determining annual heartworm transmission through effects on vector abundance. This conclusion was based on three positive pathways: (1) between precipitation and host-seeking *Oc. sierrensis* abundance, (2) between host-seeking *Oc. sierrensis* abundance and annual heartworm transmission, and (3) directly between precipitation and annual heartworm transmission. The third pathway was especially informative given that we found a significant positive correlation between abundance of host-seeking *An. punctipennis*, another vector, and annual transmission but not between precipitation and abundance of host-seeking *An. punctipennis*. It is possible that our failure to find a significantly positive correlation between precipitation and abundance of host-seeking *An. punctipennis* was due to low power, as our sample size was low; elsewhere, abundance of this species responded positively to precipitation (Vandyk and Rowley 1995). In either case, *Oc. sierrensis* were caught in numbers > 50 times greater than those of *An. punctipennis* at both PV (this study) and HREC (Lee 1971, Sacks 2002), suggesting *Oc. sierrensis* was the principle vector in the region. Regardless of the relative strengths of pathways, precipitation was strongly linked to transmission, with 58% of the variance in annual transmission explained by precipitation. These findings underscore the importance of treating pets preventively during summers following

Fig. 6. Seasonal patterns of abundance of female host-seeking mosquitoes caught in Fay/CO<sub>2</sub> traps at the PV site, indicated by 14-day running averages of data combined across years, Mendocino County, California, USA, 1995–2001. Grey areas indicate the ranges from 1995–2001 of first and last days of HDU seasons. Numbers indicate total captures in 438 trap nights. Two *Culex erythrothorax* were caught (not shown).



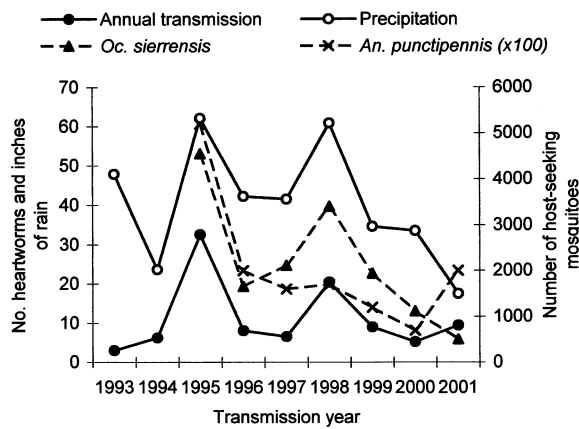


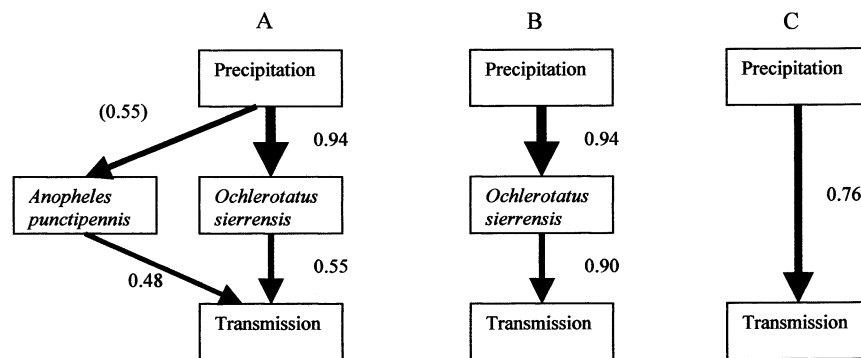
Fig. 7. Annual transmission (average annual numbers of heartworms infecting coyotes), annual precipitation, and annual catches of 2 species of mosquitoes in 2 Fay/CO<sub>2</sub> traps at the PV site, Mendocino County, California, USA.

worms live up to 7.5 years in their canid hosts, so their survival is largely dependent on host survival (Newton 1968). Although there is evidence that coyote abundance also is positively correlated with rainfall in California, the response has a one to two year lag and probably reflects effects on reproduction more so than on survival (Cypher et al. 2000, B. N. Sacks unpubl.). However, when low-precipitation years accumulate, such as during a 6-year drought, the inhibitory effects on transmission may translate to significant reductions in heartworm abundance and prevalence (Sacks 1998, Theis et al. 1999). Future studies are needed to more completely characterize the population dynamics of heartworm in coyotes and assess the magnitude of precipitation effects, including investigation of intensity-dependent effects of heartworm on survival and reproduction of coyotes, mosquitoes, and heartworms (Anderson and May 1978, May and Anderson 1978, Dye and Williams 1995).

high-rainfall years in California and, depending on local vector fauna, in Mediterranean ecosystems of Europe, Australasia, South America, and Africa.

To conclude, it is useful to place findings in the context of the total population dynamics of heartworm. Despite the destabilizing effects of precipitation on heartworm recruitment, adult heartworm abundance (prevalence × intensity) and especially prevalence were relatively constant over time (Sacks and Caswell-Chen 2003), probably owing to the stabilizing effects of adult heartworm survival on the population dynamics. Heart-

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Strength of *Oc. sierrensis* pathway assuming *An. punctipennis* is unrelated to precipitation but affects transmission =  $0.94 \times 0.55 = 0.52$

Strength of 2-vector pathway =  $0.94 \times 0.55 + 0.55 \times 0.48 = 0.78$

Strength of *Oc. sierrensis* pathway assuming *An. punctipennis* is negligible =  $0.94 \times 0.90 = 0.85$

Fig. 8. Path diagrams illustrating putative relationships and their strengths between variables linking precipitation to heartworm transmission (A, B) and, for comparison, directly between precipitation and transmission (C). Strengths are represented by standardized regression coefficients; the value contained in parentheses was not statistically significant. Standardized coefficients were calculated assuming *Anopheles punctipennis* was an important vector, which may or may not have related to precipitation (A) and assuming *Anopheles punctipennis* was a negligible vector (B).

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